The intracellular signaling pathways governing macrophage activation and function in human atherosclerosis

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Abstract

Atherosclerosis is a chronic inflammatory disease characterized by lipid accumulation and plaque formation in arterial vessel walls. Atherosclerotic plaques narrow the arterial lumen to increase the risk of heart attacks, ischemic stroke and peripheral vascular disease, which are major and worldwide health and economic burdens. Macrophage accumulation within plaques is characteristic of all stages of atherosclerosis and their presence is a potential marker of disease activity and plaque stability. Macrophages engulf lipids and modified lipoproteins to form foam cells that express pro-inflammatory and chemotactic effector molecules, stress inducing factors and reactive oxygen species. They control plaque stability and rupture through secretion of metalloproteinases and extracellular matrix degradation. Although macrophages can worsen disease by propagating inflammation, they can stabilize atherosclerotic plaques through tissue remodelling, promoting formation of a fibrous cap, clearing apoptotic cells to prevent necrotic core formation and through vascular repair. In atherosclerosis, macrophages respond to dyslipidaemia, cytokines, dying cells, metabolic factors, lipids, physical stimuli and epigenetic factors and exhibit heterogeneity in their activation depending on the stimuli they receive. Understanding these signals and the pathways driving macrophage function within developing and established plaques and how they can be pharmacologically modulated, represents a strategy for the prevention and treatment of atherosclerosis. This review focusses on the current understanding of factors controlling macrophage heterogeneity and function in atherosclerosis. Particular attention is given to the macrophage intracellular signaling pathways and transcription factors activated by biochemical and biophysical stimuli within plaques, and how they are integrated to regulate plaque formation and stability.

Introduction

Atherosclerosis is a key factor underlying cardiovascular disease, a major cause of death in the Western world [1, 2]. It is a chronic inflammatory condition that attracts cells of the innate and adaptive immune systems into plaques [3]. Macrophages are present at all stages of atherogenesis where they impact lesion development [3]. In the early stages of plaque development, monocytes are recruited to the activated endothelium and transverse across it in response to chemoattractants released following endothelial injury by abnormal lipid levels and flow perturbation [4]. The monocytes differentiate to macrophages that engulf oxidized low density lipoproteins and transform into foam cells that are retained in the vessel wall. These become lipid engorged and eventually die thus augmenting local inflammation. In advanced plaques it has also been shown that as well as monocyte recruitment, the increase in macrophage numbers may result from local proliferation [5]. Activated plaque macrophages secrete cytokines, chemokines and toxic oxygen radicals that further direct and amplify the local immune response. This increases the volume of the intima, forming atherosclerotic plaques and leading to narrowing of the blood vessels. Macrophages can destabilise the plaque resulting in plaque rupture and thrombosis through release of proteases and tissue factor, respectively. Plaques tend to rupture at sites of increased macrophage content [6]. These events highlight the destructive functions of macrophages and their strong contribution to atherogenesis. However, it is well established that macrophages can resolve inflammation and stabilise plaques through secretion of resolving mediators, anti-inflammatory cytokines, and extracellular matrix components and by remodelling and restoring inflamed or injured tissue [7]. Macrophages are professional phagocytes and can uptake excess lipids, as well as remove apoptotic cells that would otherwise die and enhance the volume of the plaque necrotic core [8].

Characterization of macrophage heterogeneity within atherosclerotic plaques

The general concept that macrophages are heterogeneous cells with M1-like pro-inflammatory macrophages playing a pivotal role in driving atherogenesis, while M2-like pro-resolving, anti-inflammatory macrophages stabilise plaques, is a simplified

notion. This idea stemmed from classification of macrophages activated by specific stimuli *in vitro* [9]. In experimental systems, the classical inflammatory macrophage phenotype, known as M1 macrophages, are induced by stimuli such as microbial products that ligate Toll-like receptors (TLR), including lipopolysaccharide and CpG, or a pro-inflammatory cytokine such as tumor necrosis factor-α (TNF) or interferon-gamma (IFNγ). M1 macrophages contribute to a strong inflammatory programme, producing pro-inflammatory cytokines and chemokines, phagocytosing and killing pathogens and in the process causing tissue destruction [9]. By contrast, M2 macrophages, polarised *in vitro* by interleukin 4 (IL-4) or IL-13, produce anti-inflammatory and pro-resolving factors, such as transforming growth factor-β (TGF-β) and IL-10, to improve tissue remodelling and repair and by clearing dying cells and debris. However, within *in vivo* environments, such as in atherosclerotic lesions, activation of macrophages is much more complex due to the diverse environmental signals simultaneously received and macrophages will be a continuum between M1 and M2 phenotypes/activation states [9]. Moreover, the nature and strength of environmental signals will change continuously over the atherogenesis process, resulting in a combination of different macrophage subtypes in the lesion depending on the maturation stage and stimuli present [10, 11].

As well as M1 and M2 macrophage classifications, several other phenotypes have been proposed to be involved in progression and regression of murine atherosclerosis, including oxidized (Mox) macrophages, hemoglobin-related (HA-mac, M(Hb), and Mhem) macrophages and M4 macrophages (see review by Li et al [12]). Single cell RNA sequencing of plaque macrophages has further classified subsets, including non-foamy and foamy macrophages and lipid metabolism-related trigger receptor expressed on myeloid cells 2 (TREM2) macrophages, also called lipid-associated macrophages [13, 14]. Foamy macrophages express a high level of TREM2, which inhibits TLR-driven pro-inflammatory factor production (such as TNF and IL-6) and inflammatory responses [15]. Non-foamy macrophages are considered more inflammatory than foamy macrophages [14]. However, more information is needed on the exact roles of all these subsets in human atherosclerosis. Likewise, it is difficult to distinguish specific activation phenotypes of macrophages within plaques due to a lack of markers that clearly delineate different states of activation.

Regardless of the different classification of human plaque macrophage phenotypes throughout the years, it still appears that there are subsets that have a more pro-inflammatory phenotype and those with a more anti-inflammatory phenotype albeit different in characteristics to the originally defined M1 and M2 *in vitro* classified macrophages. Inflammatory macrophages are the predominant population of the human plaque shoulder regions and are associated with instability [16]. It is also worth mentioning that while M2 like macrophages are generally thought of as anti-atherogenic, M2 macrophages can also have pro-atherosclerotic properties. For example, $CD163^+$ M2-like macrophages, due to their functions, could promote intraplaque angiogenesis, vascular permeability, and leukocyte infiltration, accelerating atherogenesis [17].

Regulation of macrophage activation and polarisation within atherosclerotic plaques

As previously highlighted, the way macrophages become activated during the evolution of atherogenesis, is a consequence of the local microenvironment. In atherosclerosis, macrophages are exposed to activating stimuli including inflammatory cytokines, oxidized lipids, cholesterol crystals, metabolic factors, hypoxia, apoptotic and necrotic cells, extracellular matrix and other factors [18]. This array of activating factors will engage specific receptors and result in the integration of multiple and often contradictory intracellular signaling pathways within the cell to define specific transcriptional responses and subsequently phenotypes and functional output (Figure 1). While much of the effects of plaque activating factors for macrophages have been analysed *in vitro*, this helps understand the processes *in vivo* and where potential targets could be identified for therapeutic intervention. The potential macrophage activating factors and the intracellular pathways they drive will be discussed.

Cytokines and intracellular signaling

Within the atherosclerotic lesions, several cytokines drive macrophage pro and anti-inflammatory functions. TNF, for example, signals through TLR4 and the downstream nuclear factor NF kappa B (NFκB) to polarize macrophage pro-inflammatory responses and promote plaque growth and instability [19]. Inhibition of NFκB activation in macrophages causes a reduction of foam cell formation by

preventing apoptosis and inflammatory responses [20]. As well as NFκB, TLR signaling also activates interferon regulatory factor-3 (IRF3) and activator protein 1 transcription factors to induce pro-inflammatory responses.

Another key pathway driven by cytokine signaling in atherosclerosis is the Janus Kinase (JAK)-Signal Transducer and Activator of Transcription (STAT) pathway. Interferons (IFNs) have been identified as essential components of atherogenesis [121]. IFNα and IFNγ both induce IFN-stimulated gene expression through JAK-dependent phosphorylation of STAT1. This activated STAT1 can synergize with the NFκB pathway to amplify pro-atherosclerotic effects. IFNγ can also induce lipid uptake, and foam cell formation.

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that has recently been targeted in atherosclerosis prevention [22]. IL-1β is a potent inducer of pro-inflammatory IL-6 and ICAM/VCAM expression in vascular smooth muscles to attract monocytes/macrophages. The CANTOS trial has highlighted the efficacy of prevention when targeting IL-1 [23]. IL-1 shares a conserved cytosolic sequence called the Toll-like/IL-1 receptor domain and engages transduction pathways that induce new gene transcription via activation of NFκB and mitogen-activated protein kinase (MAPK) that regulate macrophage inflammation, lipid accumulation and plaque formation [24, 25].

IL-6 activates STAT1 and STAT3 and generally drives inflammatory events. Sustaining STAT1/STAT3 activation aggravates lesion development and IL-6 has been another target cytokine in atherosclerosis prevention [26]. IL-4 and IL-13 promote M2-like polarization through activation of STAT6, but also IRF4, and peroxisome proliferator-activated receptor γ (PPAR-γ) to downregulate pro-inflammatory responses [27]. Ligation of the IL-10 receptor with IL-10 activates STAT3 [28]. Therapeutic delivery of IL-10 has been shown to suppress atherosclerosis due to STAT3 activity which downregulates NFκB signaling and pro-inflammatory effects [29]. IL-10, IL-4 and TGF-β can also signal via phosphoinositide 3-kinase (PI3K)/Akt signaling pathways [30,31] that play a crucial role in the survival, proliferation, and migration of macrophages and contribute to anti-inflammatory effects. TGF-β increased cholesterol efflux and attenuated foam cell formation in

ApoE−/− mice that constitute an experimental model of atherosclerosis [32]. IL-10 and TGF-β also activate AMP-activated protein kinase (AMPK), a key regulator of cell metabolism, inducing M2-like macrophage functions and facilitating fatty acid oxidation [33]. Chemokines are a group of secreted proteins within the cytokine family which assist entry of monocytes into plaques and regulate macrophage phenotypes. Chemokines can also signal through NFκB, MAPKs and PI3K/AKT pathways as well as g-protein coupled receptor signaling. Chemokine and chemokine receptor knockout in atherosclerosis models has highlighted the importance of chemokines in plaque development, as reviewed by Georgakis et al [34].

Thus, key intracellular signaling pathways driven by cytokines are NFκB, STAT1, IRFs and MAPKs driving pro-inflammatory macrophages and STAT3, STAT6, PPARγ and PI3K/AKT promoting anti-inflammatory pathways. These pathways are also activated by stimuli other than cytokines that present in the atherosclerotic plaques.

Metabolic factors

Recent studies have revealed a complex metabolic environment within atherosclerotic plaques and metabolism and metabolic factors play a major role in macrophage polarization and function (reviewed by [35, 36]). Metabolic pathways influenced by inflammatory stimuli and signaling include the tricarboxylic acid cycle, aerobic glycolysis, oxidative phosphorylation (OXPHOS), the pentose phosphate pathway and fatty acid oxidation. Upon activation in a pro-inflammatory microenvironment, e.g., by oxidized LDL, cholesterol crystals or inflammatory cytokines, macrophages undergo a metabolic switch to upregulate aerobic glycolysis over OXPHOS, which enables them to meet the energy demands required to counteract the dysregulated environment [37, 38]. This results in increased expression of the transcription factor, hypoxia-inducible factor-1α (HIF1α), which facilitates enhanced production of glycolytic enzymes and pro-inflammatory cytokines including IL-1β and IL-6 that further contribute to inflammation [39]. TLR4 activation and IFNγ, NFκB and Akt signaling all trigger key metabolic alterations in macrophages, including increased glucose uptake [40] and increased aerobic glycolysis flow towards lactate, an intermediate for anabolic reactions that have been

linked to pro-inflammatory responses. Increased glycolysis uncouples the mitochondrial electron transport chain from ATP synthesis, increasing the pentose phosphate pathway and NADPH oxidase activity resulting in increased ROS that leads to plaque instability [41]. Hypoxia also increases glycolysis through HIF1α as part of a network of transcription factors regulating cellular metabolism. Cellular studies have revealed that increased expression of glycolytic genes correlates with increased plaque vulnerability and inflammation [42].

By contrast, macrophages with anti-inflammatory functions tend to rely more on OXPHOS to generate ATP in a sustained manner to support the resolution of inflammation. IL-4 or fatty acid stimulated macrophages, for example, induce OXPHOS which is dependent on peroxisome proliferator-activated receptors (PPARs) and their co-activator peroxisome proliferator-activated receptor gamma co-activator 1 to increase mitochondrial biogenesis and respiratory capacity [43] and increase uptake and use of fatty acids in β-oxidation. This is generated through increased STAT6 and PPARγ-induced gene expression. Foamy macrophages in atherosclerotic lesions have enhanced expression of OXPHOS-related genes and PPARγ signaling signatures. The macrophage energy sensor, AMPK, is also upregulated through PPARγ to drive intracellular signaling of metabolic pathways to polarise to this anti-inflammatory phenotype, while suppressing pro-inflammatory responses [44]. The mammalian target of rapamycin (mTOR) is another metabolic sensor that regulates lipid metabolism, promoting fatty acid and triglyceride synthesis and decreasing fatty acid oxidation and lipolysis, leading to cellular lipid accumulation and advancing foam cell formation [36].

Epigenetic programming

Changes in macrophage metabolism promote epigenetic changes that regulate plaque advancement or plaque stability. These changes in gene expression are predominantly induced through DNA methylation, post-translational histone modifications and noncoding RNA. DNA methylation results from transfer of methyl groups to cystine residues in DNA, by DNA methyltransferases. It is now considered that DNA methylation is a key epigenetic mechanism in the pathogenesis of atherosclerosis [45]. The DNA methyltransferases-PPARγ pathway in macrophages,

for example, regulates chronic inflammation and atherosclerosis development in mice [46].

When nucleosomes are tightly packed, transcription is inhibited as the transcriptional machinery required cannot be accessed. Histone modifying enzymes add or remove acetyl or methyl groups to control access and transcription. Addition of these groups plays a role in cholesterol metabolism, for example H3K27 inhibits cholesterol efflux by modification of ABCA1, therefore accelerates foam cell formation [47]. The histone demethylase, JMJD3, alters the polarization of macrophages to a more anti-inflammatory phenotype and its absence in macrophages accelerates atherosclerosis [48]. Histone methylation after an initial exposure of oxidized LDL can induce a long-lasting epigenetically-retained memory that impacts the cells response to a second stimulus. This epigenetic change is known as trained immunity and has been shown to impact atherosclerosis progression [see reviews 49, 50]. For a full overview on epigenetic regulations of macrophages in atherosclerosis we refer the reader to a review by Yang et al [51].

MicroRNAs (miRs) and long noncoding RNAs control post translational regulation of gene expression. Many miRs have been implicated in inhibiting cholesterol efflux through targeting ABCA1 or inhibiting cytokine release and uptake of lipids in macrophages [52]. It has been shown that miR-33 drives macrophages to a pro-inflammatory state through alterations in metabolism, while miR-155 skews macrophage pro-inflammatory responses by repressing negative regulators of inflammatory cytokines [53, 54]. miR-23a-5p expression is positively correlated with plaque progression and vulnerability due to effects on ABC transporters [55]. A number of long noncoding RNAs have also been found to exert regulatory roles on the macrophage metabolism and macrophage plasticity, consequently promoting or suppressing atherosclerotic inflammation, as reviewed in [56].

Lipid uptake and metabolism

Lipid uptake into macrophages is key to foam cell formation and atherogenesis. Lipid uptake by macrophages is an early, and ongoing, step in the development of

atherosclerotic plaques This uptake of lipid can have both pro- or anti-inflammatory effects, depending on the type of lipid and balance of transcription factor activation. Uptake of excess cholesterol or other oxysterols into foam cells will, via PPARγ activation, stimulate transcription of retinoid X receptor (RXR) and liver X receptor (LXR), resulting in the expression of ATP-binding transporters (ABCA1 and ABCG1) [57] that increase cellular cholesterol efflux. This will suppress inflammation and plaque formation. However, modified LDL or cholesterol crystals, amplifies TLR signaling and activates the nucleotide-binding domain leucine-rich repeat (NLR) and pyrin domain containing receptor 3 (NLRP3) inflammasome, resulting in pyroptosis and increased pro-inflammatory IL-1β and IL-18 synthesis [58]. Inflammasome activation is a special form of inflammation participating in the onset and development of atherosclerosis [59]. Exposure to atherogenic oxidized phospholipids also leads to a pro-inflammatory phenotype, mediated by TLR2 and the transcription factor nuclear erythroid 2-related factor 2 (nrf2) [60]. Most saturated fatty acids in plaques initiate inflammatory responses via signaling through TLR2 and TLR4. However, activation of PPARγ by omega-3 unsaturated fatty acids, can antagonize the activities of the transcription factors STAT1 and NFκB, enhancing OXPHOS and contributing to macrophage anti-inflammatory effects.

Efferocytosis

High levels of ox-LDL and cholesterol overload in macrophage results in their apoptosis. Efferocytosis is a process in which macrophages remove programmed dead apoptotic cells to clear them from forming plaques [61] slowing early development of atherosclerosis. Uptake of apoptotic cells are known to skew macrophage functions to a more M2-like pro-resolving phenotype and further enhance uptake. At the initial stage of the development of lesions, macrophage apoptosis and efferocytosis is protective for removing lipid overloaded cells. However, the excess load of apoptotic cells and a pro-inflammatory environment in advanced plaques, impacts the phenotype and phagocytic efficacy of macrophages. Defects in efferocytosis, seen in later stages of plaque development, lead to reduced clearance of apoptotic macrophages, secondary necrosis with release of immunogenic/pro-inflammatory cytoplasmic stimuli, expansion of necrotic cores and plaque instability [62]. The engulfment step in efferocytosis through

phosphatidylserine receptors, MerTK or integrins activates signaling pathways associated with cytoskeletal rearrangement via small GTPase signaling cascades, that remodel dynamic actin and drive uptake. To mount an effective efferocytic response, macrophages alter their metabolic profile to favour OXPHOS and activation of the phagolysosome to degrade engulfed apoptotic cells. Several pathways in anti-inflammatory, pro-resolving pathways can enhance efferocytosis within the atherosclerotic plaque, including the PPARγ pathway [63]. The effects of efferocytosis in atherosclerosis are review further by Wang et al [64].

Physical stimuli and mechanotransduction in macrophage activation and atherogenesis

As well as cytokines, metabolic factors, modified lipids and their uptake, it is now well recognised that macrophages can respond to their physical environment. Macrophage functions are known to be influenced by extracellular matrix composition, topography and stiffness, stretch and shear stress, flow and charge [65]. These stimuli are constantly changing during atherogenesis, for example, the stiffness of aorta and extracellular matrix composition is changed with atherosclerosis progression. Within the plaque, in areas of lower collagen content, macrophages tend to exhibit a more pro-inflammatory phenotype while higher collagen content (higher stability plaques) results in more anti-inflammatory macrophages [66]. The collagen matrix can also modulate lipid uptake and metalloproteinase secretion of macrophages [67]. These effects are brought about by mechanosensing and specific mechanosensitive receptors e.g., transient receptor potential cation channels (TRPV) and Piezo channels (for review see [68]). High matrix stiffness and low shear stress in vessel walls primes macrophages toward a pro-inflammatory M1-like phenotype, whereas oscillatory shear stress primes macrophages toward an anti-inflammatory M2-like phenotype. Shear stress can also impact macrophage phagocytic clearance and expression of matrix metalloproteinases that degrade matrix and leading to plaque instability [69, 70]. The signaling pathways downstream of mechanosensors in macrophages are still being unravelled, although it is thought oxidized LDL can activate TRPV1 to increase calcium and activate LXRs, while matrix stiffness sensed by sTRPV4 increases uptake of oxidized LDL [68]. Piezo 1 can drive pro-inflammatory

mediators including TNF, IL-1 and IL-6 [71, 72]. Hippo can affect the phenotype of macrophages by sensing internal and external mechanical stimuli [73]. Cell-adhesion and integrin engagement in macrophages recruits an array of adaptor and signaling proteins to transduce mechanical signals, e.g., the inflammasome pathway is regulated by cytoskeleton-mediated mechanotransduction. Cell-adhesion based mechanotransduction activates ASC by Pyk2 and FAK to enhance NLRP3, NLRC4, and AIM2 activity, whereas cell adhesion can inhibit the pyrin inflammasome through activation of the GTPase RhoA via FAK [74].

Conclusions

Macrophage function is critical in regulating atherogenesis and plaque stability. The factors present in the developing and advanced plaque environment provide a plethora of signals that are integrated within cells to drive output. Understanding the range of stimuli, signaling pathways and activated transcription factors, and how they are integrated is pivotal for devising strategies for their modification to regulate development of the plaque and to resolve the atherogenesis process. Several types of microenvironmental stimuli orchestrate macrophage function in atherosclerotic plaques and here we have highlighted the effects of cytokines, metabolic factors, lipids, efferocytosis regulators, mechanostimuli and epigenetic programming. All these will undergo crosstalk to determine whether localized plaque macrophages progress atherogenesis. While we are making progress in unravelling how macrophages are regulated in atherogenesis, the heterogeneity and complexity of macrophages and how their response to microenvironmental changes over time needs to be further clarified to ensure future targets for the disease are maximised.

Perspectives

Importance of the field: Macrophages are a key feature in atherosclerosis where they become activated by a plethora of microenvironmental stimuli and develop heterogeneous phenotypes and functions that significantly contribute to plaque development and instability.

Current thinking: Blocking inflammatory pathways e.g., IL-1β by canakinumab, has clinical benefits, highlighting the importance of immune cell regulation in atherosclerosis treatment. Simultaneously regulating other macrophage intracellular signaling pathways to downregulate inflammation, enhance resolution and increase plaque stability, could provide a more effective therapy.

Future directions and challenges: A key challenge in developing such therapies is that macrophages must integrate all intracellular signals received within the developing plaque. If more effective therapies targeting macrophages are to be devised, further research is needed to fully understand the molecular signaling mechanisms and how they synergise or antagonise macrophage activation to halt or resolve atherosclerosis without influencing other key homeostatic roles such as infection control.

Abbreviations

AMPK	AMP-activated protein kinase
HIF1α	hypoxia-inducible factor-1α
IFNγ	interferon-gamma
IL-4	interleukin 4
IRF3	interferon regulatory factor-3
LDL	low-density lipoprotein
LDLR	LDL receptor
LXR	liver X receptor
NLR	nucleotide-binding domain leucine-rich repeat
miRs	MicroRNAs
MAPK	mitogen-activated protein kinase
NFκB	nuclear factor kappa B
OXPHOS	oxidative phosphorylation
PI3K	phosphoinositide 3-kinases
PPAR-γ	peroxisome proliferator-activated receptor γ
NLRP3	nucleotide-binding domain leucine-rich repeat and pyrin domain containing receptor 3
ROS	reactive oxygen species
RXR	retinoid X receptor
STAT	signal transducer and activator of transcription
TGF-β	transforming growth factor-beta
TRPV	transient receptor potential cation channels
TLR	Toll-like receptor
TNF	tumor necrosis factor-α
TREM2	Triggering receptor expressed on myeloid cells 2

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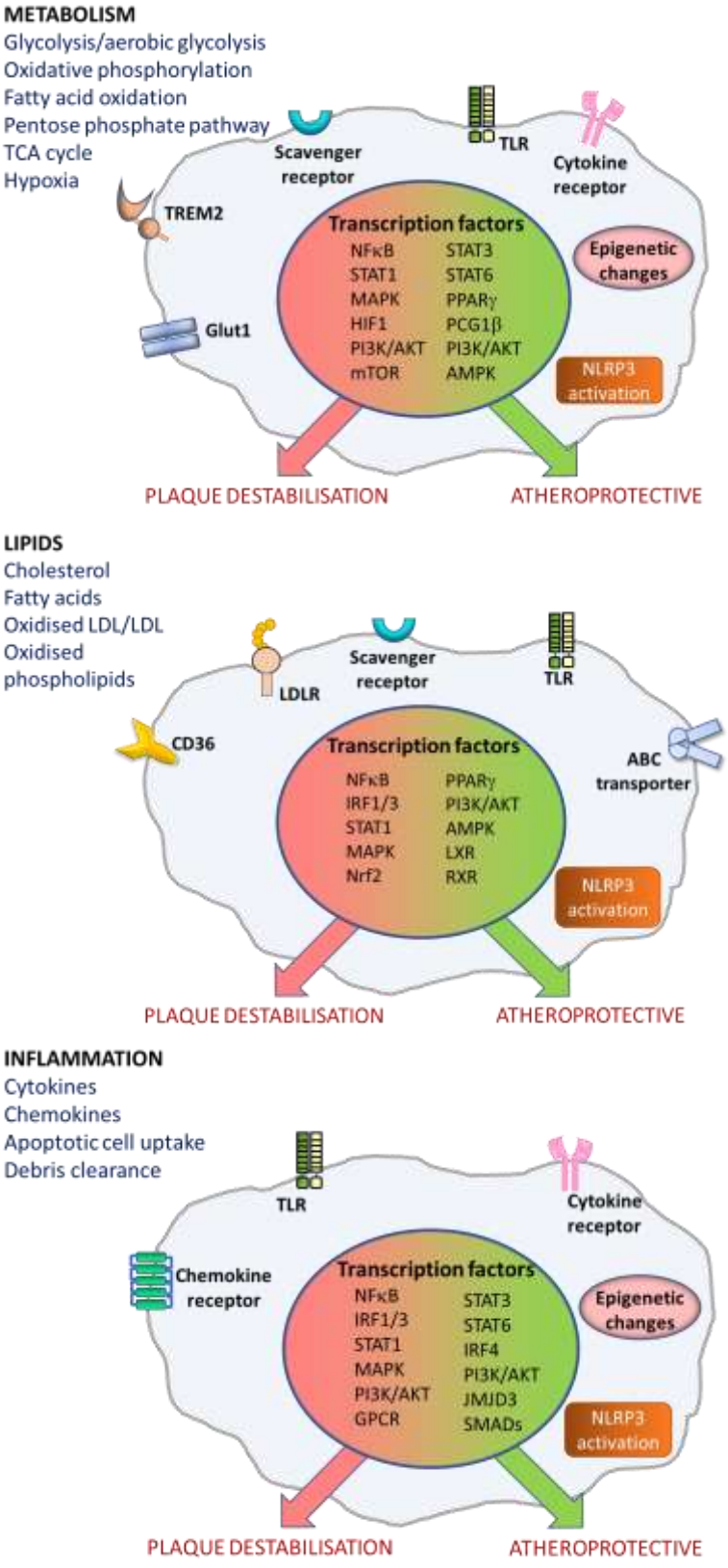
Figure legend

Figure 1. Microenvironmental stimuli and signaling pathways controlling macrophage activation and function in atherosclerosis. Macrophages within the developing and advanced atherosclerotic plaques are exposed to a multitude of stimuli including metabolic factors, lipids and other inflammatory mediators. Examples of receptors that are engaged and transcription factors activated are illustrated for metabolic factor, lipid and inflammatory mediator stimuli are shown. Activation of macrophages through these signalling pathways drive specific functions that lead to plaque destabilisation through production of pro-inflammatory cytokines and reactive oxygen species, lipid accumulation, matrix degradation and cell necrosis, or are atheroprotective through anti-inflammatory cytokine production, tissue remodelling/repair, cholesterol efflux, efferocytosis and regulation of plaque cap stability. The NLRP3 inflammasome can be activated by many stimuli and plays an important role in the pathogenesis and progression of atherosclerosis. Epigenetic events also change the course of atherosclerosis.

Figure 1


METABOLISM
Glycolysis/aerobic glycolysis
Oxidative phosphorylation
Fatty acid oxidation
Pentose phosphate pathway
TCA cycle
Hypoxia
Scavenger receptor
TLR
Cytokine receptor
TREM2
Transcription factors
NFκB
STAT1
MAPK
HIF1
PI3K/AKT
mTOR
STAT3
STAT6
PPARγ
PCG1β
PI3K/AKT
AMPK
Epigenetic changes
Glut1
NLRP3 activation
PLAQUE DESTABILISATION
ATHEROPROTECTIVE
LIPIDS
Cholesterol
Fatty acids
Oxidised LDL/LDL
Oxidised phospholipids
LDLR
Scavenger receptor
TLR
CD36
Transcription factors
NFκB
IRF1/3
STAT1
MAPK
Nrf2
PPARγ
PI3K/AKT
AMPK
LXR
RXR
ABC transporter
NLRP3 activation
PLAQUE DESTABILISATION
ATHEROPROTECTIVE
INFLAMMATION
Cytokines
Chemokines
Apoptotic cell uptake
Debris clearance
TLR
Cytokine receptor
Chemokine receptor
Transcription factors
NFκB
IRF1/3
STAT1
MAPK
PI3K/AKT
GPCR
STAT3
STAT6
IRF4
PI3K/AKT
JMJD3
SMADs
Epigenetic changes
NLRP3 activation
PLAQUE DESTABILISATION
ATHEROPROTECTIVE