Cytokine 122 (2019) 154175

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Regulation of pro- and anti-atherogenic cytokines

Mitali Ray, Michael V. Autieri*

Department of Physiology, Independence Blue Cross Cardiovascular Research Center, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 19140, USA

ARTICLE INFO

Keywords:
Atherosclerosis
Inflammation
Cytokines
RNA binding protein

ABSTRACT

Despite advances in prevention and treatment, vascular diseases continue to account for significant morbidity and mortality in the developed world. Incidence is expected to worsen as the number of patients with common co-morbidities linked with atherosclerotic vascular disease, such as obesity and diabetes, continues to increase, reaching epidemic proportions. Atherosclerosis is a lipid-driven vascular inflammatory disease involving multiple cell types in various stages of inflammation, activation, apoptosis, and necrosis. One commonality among these cell types is that they are activated and communicate with each other in a paracrine fashion via a complex network of cytokines. Cytokines mediate atherogenesis by stimulating expression of numerous proteins necessary for induction of a host of cellular responses, including inflammation, extravasation, proliferation, apoptosis, and matrix production. Cytokine expression is regulated by a number of transcriptional and post-transcriptional mechanisms. In this context, proteins that control and fine-tune cytokine expression can be considered key players in development of atherosclerosis and also represent targets for rational drug therapy to combat this disease. This review will describe the cellular and molecular mechanisms that drive atherosclerotic plaque progression and present key cytokines that participate in this process. We will also describe RNA binding proteins that mediate cytokine mRNA stability and regulate cytokine abundance. Identification and characterization of the cytokines and proteins that regulate their abundance are essential to our ability to identify therapeutic approaches to ameliorate atherosclerotic vascular disease.

1. Introduction

Cardiovascular disease is the leading cause of death worldwide, with 1 in 5 deaths annually. Despite increased awareness in the general public about how lifestyle choices affect vascular health, cardiovascular disease remains the number one cause of death both nation and worldwide to date. It is widely recognized that atherosclerosis, the underlying cause of coronary heart disease, peripheral vascular disease, and stroke, is the primary contributor to the majority of all cardiovascular diseases [1]. Vascular disease can affect the entire body as arteries and veins are responsible for nourishing the body in its entirety. Roughly 92.1 million individuals are affected by at least one type of vascular disease in the United States alone [1]. As the developed world adopts a more sedentary, inactive lifestyle, atherosclerotic vascular disease itself accounts for significant morbidity and mortality. Controllable risk factors for vascular disease include obesity, smoking, physical inactivity, hypertension, stress, high LDL levels, and uncontrolled diabetes. Disease prevention focuses on healthy lifestyle choices that limit these risk factors. The incidence of atherosclerosis and other vascular diseases increases as the number of patients with co-morbidities such as obesity, metabolic syndrome, and diabetes mellitus grows, along with an aging population in the developed world.

2. Development of atherosclerosis

Blood vessels are composed of three layers: an outermost layer or adventitia, a middle layer or media, which is composed of vascular smooth muscle cells (VSMCs), and an innermost layer called the intima. The intima is formed by simple squamous endothelial cells (ECs) and contains the endothelium, which is the interface between the vessel wall and circulating blood. Atherosclerotic lesions typically develop in large and medium-sized arteries and can lead to ischemia in areas including the heart and brain or peripheral arterial disease in the extremities [2]. Numerous cells from each of these layers participate in the vascular inflammation indicative of atherosclerosis. ECs, VSMCs, and infiltrating immune cells communicate with each other bidirectionally through the production and secretion of cytokines and production of cytokine receptors [8]. Plaque rupture can result in myocardial infarction, stroke, or thrombosis, which are often lethal. Atherosclerosis is a lipid-driven, chronic vascular inflammatory disease

* Corresponding author at: Department of Physiology, Independence Blue Cross Cardiovascular Research Center, Temple University School of Medicine, Room 1050, MERB 3500 N. Broad St., Philadelphia, PA 19140, USA.
E-mail address: mautieri@temple.edu (M.V. Autieri).

https://doi.org/10.1016/j.cyto.2017.09.031
Received 9 June 2017; Received in revised form 29 August 2017; Accepted 26 September 2017
Available online 06 December 2017

that develops in response to various insults to the vasculature such as oxidative stress in the form of oxidized excess low-density lipoprotein (oxLDL). The lipid theory of atherogenesis states that oxidation of low-density lipoprotein (LDL), one of the earliest initiation factors of atherosclerosis, occurs as a normal metabolic consequence. Oxidation of LDL exposes numerous epitopes and excess oxLDL acts as an antigenic, pro-inflammatory factor [3,4]. Internalization of oxLDL results in a series of dramatic events, including activation of Nuclear Factor Kappa B (NF-κB), a "master switch" for transactivation of many cytokine genes that drive the cellular atherogenic response. Several studies have shown that oxLDL stimulation of ECs, VSMCs, and leukocytes leads to expression of pro-inflammatory cytokines [3]. While the lipid theory has become both well established and accepted, unmodified or "native" LDL has also been implicated as a trigger of inflammation and atherogenesis [4,5]. Studies demonstrate that unmodified LDL, specifically Apolipoprotein B100 (ApoB100), the primary protein constituent of LDL, results in expression of pro-inflammatory cytokines as well as activate Mitogen Activated Protein Kinase (MAPK) and calcium signaling [6]. The majority of these experiments focus specifically on T cell activation and explore vaccination as a potential therapy, though there are additional studies investigating the effects of ApoB100 in additional cell types including macrophages, platelets, and dendritic cells [4,7–9].

The endothelium in healthy individuals maintains a non-thrombogenic, non-adherent surface; it maintains vascular tone by synthesis of vascular dilatory and constricting molecules; acts as a permeability barrier for exchange of substances into artery wall; and regulates lipid modification as it is transported into the artery wall. Atherosclerosis begins with EC dysfunction, which is initiated by oxLDL induced expression of TNFα, a master pro-inflammatory cytokine. TNFα in turn induces expression of additional pro-inflammatory cytokines and cell adhesion molecules (CAMs). The discovery that increased expression of CAMs leads to increased endothelial permeability and leukocyte extravasation gave rise to the inflammatory hypothesis of atherogenesis, initially proposed by Russell Ross. This hypothesis suggests that immune cell adhesion leads to an activated, or "inflamed" endothelium, which is the earliest cellular event in development of atherosclerosis [7]. The earliest type of atherosclerotic lesion is termed the "fatty streak", which is not uncommon in most children [10]. This initial lesion is purely inflammatory as it consists of only monocyte-derived macrophages and T cells [11]. As inflammation persists, additional monocytes are recruited into the subendothelial space, differentiate into macrophages, and rapidly uptake oxLDL via scavenger receptors. As macrophages continue to scavenge for oxLDL they can become overloaded and become "foam cells", which are incapable of leaving the artery and continue to actively secrete pro-inflammatory cytokines, fueling ongoing inflammation [2,12]. As plaque development progresses to intermediate lesions, macrophages and foam cells become apoptotic. In early atherogenesis additional macrophages are able to clear the apoptotic cells, however in later pathogenesis, apoptotic macrophages and foam cells accumulate and form a necrotic core, which is a feature of later stage, complex atherosclerotic lesions.

Pro-inflammatory cytokines also cause VSMCs to undergo phenotypic switching during atherogenesis, specifically in the intermediate lesion stage. VSMCs transform from their normally differentiated, contractile phenotype to an active "synthetic" state. Synthetic VSMCs are able to migrate from the media into the intima and proliferate, phagocytize oxLDL, and secrete additional pro-inflammatory cytokines, which in turn, recruit additional VSMCs and immune cells. VSMCs also produce matrix, which retains lipids and the inflammatory cell types within the lesion. Eventually intermediate lesions can develop into fibrous plaques or advanced lesions, which typically lead to clinical manifestations. These advanced lesions are comprised of a necrotic core of apoptotic foam cells and T cells, covered in a thick layer of VSMCs that have formed a fibrous cap, consisting of collagen and connective tissue. Plaque vulnerability is dependent upon the thickness of the fibrous cap. As inflammation persists, pro-inflammatory cytokines are able to secrete matrix metalloproteinases (MMPs) that degrade the matrix, leading to thinning of the fibrous cap. Plaque rupture typically occurs as a consequence of decreased cap integrity, leading to myocardial infarction (MI) or stroke. Plaque rupture from a fractured cap may also lead to thrombus formation, which is the major cause of vascular mortality in humans.

3. Cytokines that participate in atherosclerosis

It is important to recognize that the microenvironment of the atherosclerotic plaque is a dynamic collection of multiple cell types including ECs, VSMCs, and infiltrating immune cells, each in various stages of activation and synthesis of cytokine products. As such, most cytokines initiate a complex and varied repertoire of responses on their target cells and can initiate, promote, and potentially resolve plaque formation. Thus, this complex microenvironment results in a complex milieu consisting of multiple cytokines with redundant and opposing effects. To better describe the actions of cytokines on atherogenesis, it is convenient to classify them into different families, typically based on structure; we can also categorize them by function, specifically into one of two groups: pro-inflammatory and anti-inflammatory. Cytokines typically act in synergy with other cytokines of the same functional group and are able to induce simultaneous cellular processes as the previously mentioned cell types have extensive overlap in which cytokines they are able to produce, secrete, and respond to. Cytokines are able to balance each other: pro-inflammatory cytokines initiate and sustain inflammation, while anti-inflammatory cytokines limit the magnitude of inflammation, typically pushing it to resolve, limiting tissue damage. Skewing of this delicate balance can result in chronic inflammation or inability to fight infection. Vascular diseases are often the consequence of such a shift and subsequent uncontrolled inflammation (see Fig. 1).

4. Pro-and anti-inflammatory cytokines

Pro- and anti-inflammatory cytokines are frequently associated with their effects on T helper cells, with pro-inflammatory cytokines being associated with T_h1 and anti-inflammatory cytokines characterizing T_h2. Polarization of T cells to T_h1 in atherosclerosis has been established in mouse models and has been suggested in humans [13,14]. Because atherosclerosis is a chronic inflammatory disease, T_h1 cytokines are much more prevalent in human atherosclerotic lesions than T_h2 cytokines. In addition to T_h1 and T_h2 are T_h17 and T regulatory (Tregs) cells which have a similar but distinct dynamic. T_h17 cells are characterized by expression of pro-inflammatory cytokine IL-17 and the ability to inhibit Tregs. Tregs, formerly called suppressor T cells, are immunosuppressive and are known to play a role in prevention of autoimmune disease as well as attenuation of atherosclerosis in mice. Tregs lymphocytes are found in human atherosclerotic plaque, and currently the subject of intense study. This lymphocyte subset is considered to be atheroprotective, as adaptive transfer of Tregs reduces the production of T_h1 cytokines including IFNγ and concurrently increases expression of the T_h2 cytokine IL-10 [15]. Not surprisingly, depletion of these cells increases atherosclerosis in mice [16].

A paradigm similar to T helper cells exists among macrophages. Pro- and anti-inflammatory cytokines are able to polarize macrophages to their classical M1 or alternative M2 phenotypes, respectively. Shifts in M1/M2 balance have been indicated in a number of inflammatory diseases, including atherosclerosis and peripheral arterial disease. Similar to the previously discussed cell types, macrophages secrete and respond to cytokines. Pro-inflammatory cytokines including TNFα and IFN-γ typically induce classically activated M1 macrophages, which in turn secrete high levels of additional pro-inflammatory cytokines including TNFα, IL-1β, IL-12, and IL-23, as well as low levels of anti-inflammatory IL-10 [17,18]. We will broadly present cytokines in terms

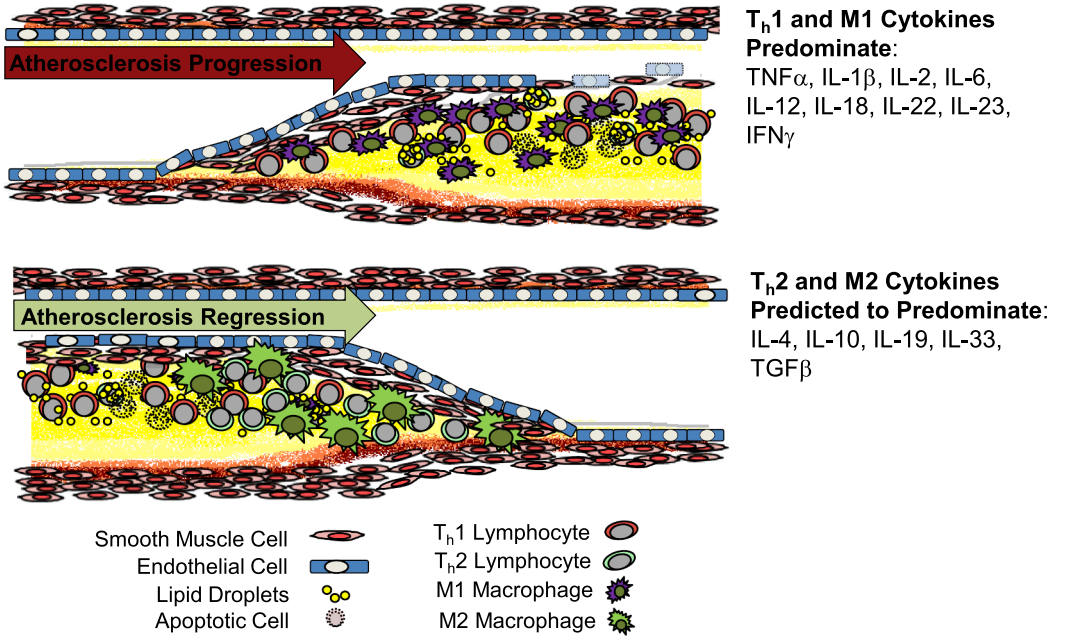

Fig. 1. Cellular and cytokine profile in atherosclerotic plaque. Pro-inflammatory T_h1 and M1 lymphocyte and macrophage derived cytokines dominate in atherogenesis, whereas anti-inflammatory T_h2 and M2 cytokines would be expected to be expressed in resolving plaque. It is hypothesized that a therapeutic goal for atherosclerosis prevention, and potentially for regression would be to tip the balance of these "opposing forces" from a predominantly T_h1 pro-inflammatory environment to a T_h2, anti-inflammatory cytokine milieu.

of their pro-atherogenic and anti-atherosclerotic properties, and briefly describe each (See Table 1).

4.1. Pro-atherogenic cytokines

Generally speaking, due to the pro-inflammatory environment of atherosclerotic plaque, pro-inflammatory, T_h1 cytokines are more prevalent in human and mouse lesions than anti-inflammatory, T_h2 cytokines. We will begin with a discussion of reported pro-atherogenic cytokines, beginning with TNFα. TNFα is considered to be a global pro-inflammatory protagonist and its post-transcriptional regulation is by far the best characterized (discussed in Section 5). While TNFα is clearly a major player in atherogenesis, we will also briefly discuss other cytokines that participate in this process.

Tumor necrosis factor alpha (TNFα) is a pleiotropic member of the tumor necrosis factor superfamily [19]. Its ability to induce other pro-inflammatory cytokines, thus amplifying its potent inflammatory effects has earned TNFα the moniker of a "master pro-inflammatory cytokine". While M1 macrophages are the primary source of TNFα in atherosclerosis, its expression can also be induced in ECs and VSMCs [20]. Induction of several pro-inflammatory cytokines and CAMs by TNFα fuels the inflammatory cascade by recruiting additional T cells and macrophages to the atherosclerotic lesion. Consistent with its effects on CAM expression, TNFα promotes leukocyte/EC interaction and extravasation in vivo, an initial step in generation of the fatty streak leading to atherosclerosis [21,22]. TNFα could potentially serve as a useful biomarker for coronary artery disease in the clinic as serum levels of TNFα correlate with early carotid atherosclerosis [20].

Our current understanding of canonical TNFα receptor signaling points to Nuclear Factor kappa B, (NF-κB), p38 MAPK, and JUN N-terminal kinase (JNK) activation as essential participants of this pathway [23]. This leads to expression of NF-κB target genes which are crucial in inflammation, cell proliferation, and response to stress [19]. Included in this list are cytokines pertinent for progression of atherosclerosis, including, but not limited to IL-1β, IL-6, IL-8, MCP-1, as well as TNFα itself [24].

Long-term stimulation of macrophages by TNFα, via a MAPK-dependent pathway downregulates macrophage scavenger receptor gene expression and protein via transcriptional and post-transcriptional processes [25,26]. These scavenger receptors play a crucial role in the reverse cholesterol pathway, as macrophages uptake and unload LDL using these receptors; exacerbated TNFa is therefore able to promote

Table 1
Cytokines and predicted atherogenicity.

Cytokine	Producer	T cell phenotype	Macrophage phenotype	Atherogenicity
IL-1β	ECs, Lymphocytes, Monocytes/Macrophages, VSMCs [34]			Pro-atherogenic [38]
IL-2	Lymphocytes [39]	Tregs [41]		Conflicting data [40,41,46]
IL-6	ECs, Lymphocytes, Monocytes/Macrophages, VSMCs [42]			Conflicting data [45,46]
IL-12	Monocytes/Macrophages, Lymphocytes [47]	T_h1 [47]		Pro-atherogenic [49,50]
IL-18	Monocytes/Macrophages [52]	T_h1 [52]		Pro-atherogenic [53–55]
IL-22	Lymphocytes, Monocytes/Macrophages, VSMCs [58]			Pro-atherogenic [59]
IL-23	Monocytes/Macrophages [60]	T_h17 [60]		Further experiments required
TNFα	Lymphocytes, Monocytes/Macrophages, VSMCs [20]		M1 [20]	Pro-atherogenic [29]
IFNγ	ECs, Lymphocytes, Monocytes/Macrophages, VSMCs [62]	T_h1 [63]	M1 [64]	Pro-atherogenic [65,66]
IL-4	ECs, Lymphocytes, Monocytes/Macrophages, VSMCs [47,74]	T_h2 [75]		Conflicting data [73,76]
IL-10	Monocytes/Macrophages, Lymphocytes [47,77]	T_h2 [47]	M2 [79]	Atheroprotective [79,81,82]
IL-19	ECs, Lymphocytes, Monocytes/Macrophages, VSMCs [84,85]	T_h2 [86]	M2 [87]	Atheroprotective [86]
IL-33	Monocytes/Macrophages, Lymphocytes [91–93]	T_h2 [92]	M2 [92]	Atheroprotective [91]
TGF β	Lymphocytes, Monocytes/Macrophages, VSMCs [47]	T_h2 [46]		Atheroprotective [94]

atherosclerosis by reducing the efficacy of this reverse cholesterol pathway. TNFα is also associated with plaque rupture as it stimulates production of MMPs and the thrombogenic protein, tissue factor, in VSMCs [27,28].

Multiple studies completed in *Tnfα*$^{-/-}$x*Apoe*$^{-/-}$ double knockout mice have found 50 to 75% decreases in atherosclerotic lesion size [29]. In studies using *Apoe*$^{-/-}$ mice treated with recombinant soluble TNFα p55 receptor, lesion size was reduced [30]. Blockade therapy targeting TNFα signaling with humanized antibody or recombinant fusion proteins has proven beneficial for patients with chronic inflammatory diseases and incidence of acute cardiovascular events is lower in arthritis patients receiving this treatment [31–33]. However, adverse effects have limited the utility of this therapy. Considering its potent pro-inflammatory, pro-thrombogenic, and anti-cholesterol clearing effects, it is not surprising that TNFα is a major pro-atherogenic cytokine. The complex molecular mechanisms that modulate TNFα abundance by regulation of its mRNA will be discussed in detail in Section 5.

Interleukin-1 beta (IL-1β) is a prototypic pro-inflammatory cytokine expressed by macrophages, ECs, and VSMCs [34]. IL-1β plays a key role in early atherogenesis. It is inflammation-responsive and induced by TNFα and it subsequently serves as a local paracrine and autocrine stimulator of several additional pro-inflammatory cytokines and CAMs, leading to immune cell extravasation and sustained local inflammation. IL-1β also promotes VSMC proliferation and migration, as well as release of MMPs [35,36]. IL-1β has been established as a pro-atherogenic cytokine in a number of mouse models. Infusion of IL-1 receptor decoy reduces fatty-streak area in *Apoe*$^{-/-}$ mice [37]. *Il1β* $^{-/-}$x*Apoe*$^{-/-}$ double knockouts also have 30% less atherosclerotic lesion size compared to *Apoe*$^{-/-}$ mice [38]. Considering its potent pro-inflammatory effects on a variety of cell types, IL-1β is a major contributor to vascular inflammatory diseases including atherosclerosis.

Interleukin-2 (IL-2) is a T lymphocyte growth factor and as such, is considered to be a pro-inflammatory T_h1 cytokine [39]. Studies have established IL-2 expression in atherosclerotic plaques, though its role has yet to be properly characterized. *In vivo* studies completed in mouse models have yielded conflicting results. One study reports *Apoe*$^{-/-}$ mice treated with IL-2 via i.p. injection while on high fat diet have increased atherosclerosis compared to controls [40]. Additionally, treatment with IL-2 antibody also decreased plaque burden in *Apoe*$^{-/-}$ mice [20]. However, another study demonstrates that IL-2 is able to attenuate atherosclerotic plaque development by activating Tregs [41]. Additional experimentation is required to further elucidate the role of IL-2 in atherogenesis.

Interleukin-6 (IL-6) is a pro-inflammatory cytokine and potent inducer of T_h17 cells [42]. Patients with unstable angina have increased plasma levels of IL-6 [43]. IL-6 is expressed in a variety of cell types involved in atherosclerosis, including macrophages, ECs, and VSMCs. IL-6 expression results in increased CAM expression in ECs, contributing to extravasation of leukocytes into the vessel wall [44]. Systemic treatment with IL-6 in *Apoe*$^{-/-}$ mice results in increased atherosclerosis, supporting the notion that IL-6 is a pro-atherosclerotic cytokine [45]. Surprisingly, *Il6*$^{-/-}$x*Apoe*$^{-/-}$ double knockout mice have increased plaque burden compared to controls [46]. Double knockout mice also have decreased leukocyte infiltration into the lesions, as well as reduced expression of MMPs, suggesting increased plaque stability. These seemingly contradictory data may be explained by the compensatory increase in expression of anti-inflammatory IL-10, as well as IL-1Rα and TNFα receptors, which may neutralize plasma levels of these inflammatory cytokines. IL-6 participation in atherogenesis is complex and has yet to be completely understood.

Interleukin-12 (IL-12) is a pro-inflammatory cytokine expressed in atherosclerotic lesions and is known to induce signal transducer and activator of transcription 4 (STAT4) and subsequently activate the transcription factor T box expressed in T cells (TBet) [47]. TBet has been classified as a marker of the T_h1 phenotype as it transactivates expression of many pro-inflammatory genes. TBet results in expression of pro-inflammatory IFNγ, which acts as a major regulator of inflammation [48]. Importantly, TBet also inhibits expression of anti-inflammatory, T_h2 cytokine IL-4. oxLDL treatment of monocytes results in IL-12 expression. Additionally, *Apoe*$^{-/-}$ mice treated with IL-12 demonstrate exacerbated atherosclerotic plaques compared to controls [49]. *Il12*$^{-/-}$x*Apoe*$^{-/-}$ mice also have decreased plaque deposition compared to mice with baseline IL-12 expression. Blockade of endogenous IL-12 via anti-IL-12 antibodies attenuates atherosclerosis in *Ldlr*$^{-/-}$ mice [50]. Studies reporting the atherogenic effects of IL-12 have focused on immune cells specifically; effects of IL-12 on vascular cells have not been reported. Thus far, IL-12 appears to be a major pro-atherogenic cytokine.

Interleukin-18 (IL-18) is a pro-inflammatory cytokine that has been shown to polarize T cells to T_h1 and induce IFNγ expression [51]. IL-18 levels are elevated in the sera of patients with coronary artery disease and IL-18 and its receptor have both been detected in human atherosclerotic plaques [52]. IL-18 is primarily secreted by macrophages and its receptor is present in macrophages, as well as ECs and VSMCs, allowing it to possibly mediate crosstalk between immune cells and the vasculature. *Il18*$^{-/-}$x*Apoe*$^{-/-}$ double knockout mice have reduced atherosclerosis compared to control mice [53,54]. Additionally, *Apoe*$^{-/-}$ mice treated with IL-18 demonstrate increased plaque burden [55]. It is interesting to note that when treated with IL-18, *Ifnγ*$^{-/-}$x*Apoe*$^{-/-}$ double knockout mice have decreased plaque burden compared to IL-18 treated *Apoe*$^{-/-}$ mice, suggesting that IL-18 and IFNγ have a synergistic relationship [53,54].

Interleukin-22 (IL-22) is a member of the IL-10 family, has been associated with T_h17, and implicated in autoimmune diseases including lupus and rheumatoid arthritis [56,57]. IL-22 expression has been found in human atherosclerotic plaques in carotid arteries and increased levels were found specifically in patients with unstable plaques [58]. IL-22 expression has also been confirmed in macrophages and T cells, as well as in VSMCs, further implicating a role in atherosclerosis. *Il22*$^{-/-}$x*Apoe*$^{-/-}$ double knockout mice fed high fat diet for 14 weeks have decreased plaque deposition compared to *Apoe*$^{-/-}$ controls [59]. Interestingly, double knockout mice in these studies also had decreased collagen levels in their plaques, suggesting the smaller plaques were less stable and IL-22 may contribute to cap thickness and plaque stability. Double knockout mice also had increased expression of contractile VSMC markers, suggesting IL-22 influences VSMC phenotypic switching to a synthetic, proliferative state.

Interleukin-23 (IL-23) is a pro-inflammatory cytokine that influences T_h17 cells [60]. Macrophages express both IL-23 and the IL-23 receptor; IL-23 is able to induce expression of several pro-inflammatory cytokines including IL-17, IL-22, and TNFα in these cells [60]. Similar to IL-22, IL-23 has been detected in autoimmune diseases including psoriasis and rheumatoid arthritis, but has yet to be extensively researched in vascular disease. A recent study investigated IL-23 in patients with carotid atherosclerosis with a specific focus on stroke [61]. The study reports significantly increased levels of IL-23 in the plasma of patients with atherosclerosis compared to healthy controls. Furthermore, during follow-up, high levels of IL-23 in the plasma was associated risk of mortality. Expression of IL-23 and the IL-23 receptor genes were markedly increased in the carotid plaques when compared to healthy vessels [61]. IL-23/LPS co-treatment of monocytes from patients with carotid atherosclerosis also resulted in increased secretion of IL-17 and TNFα compared to monocytes from healthy controls. Although these reports suggest a pro-atherosclerotic role, further studies are necessary to define the exact role for IL-23 in development of atherosclerosis.

Interferon gamma (IFNγ) is highly expressed by multiple cell types in atherosclerotic plaque and its role throughout atherogenesis continues to be extensively researched [62]. Studies focusing on IFNγ unanimously characterize it as a robust pro-atherosclerotic cytokine for several reasons. IFNγ production by T_h1 cells is able to stimulate macrophages to further secrete pro-inflammatory cytokines [63]. IFNγ

also promotes oxLDL uptake in both macrophages and VSMCs, promoting foam cell development [64]. Systemic treatment with IFNγ via i.p. injection exacerbates plaque deposition in *Apoe*$^{-/-}$ mice [65]. IFNγ treatment also results in decreased VSMC proliferation and subsequent collagen deposition in the plaque cap, suggesting it may compromise plaque stability. Reduced plaque size is observed in *Ifnγ*$^{-/-}$x*Apoe*$^{-/-}$ double knockout mice, and gene transfer of a secreted IFNγ receptor decoy in *Apoe*$^{-/-}$ mice resulted in decreased atherosclerotic plaque burden [65,66].

4.2. Anti-atherogenic cytokines

Cytokine abundance in atherosclerotic plaque is overwhelmingly T_h1 oriented [67,68]. While expressed to a much lesser degree compared to pro-inflammatory cytokines, anti-inflammatory cytokines associated with T_h2 and M2 cells are present in atherosclerotic plaques. Overexpression of T_h2 cytokines has been proposed to attenuate other inflammatory conditions such as rheumatoid arthritis, colitis, and asthma; therefore, they may also prove attractive as a therapeutic approach for atherosclerosis. Polarization of inflammatory cells to anti-inflammatory or reparative phenotypes could serve as a promising therapeutic avenue for atherosclerosis. One therapeutic goal for regression of existing atherosclerotic plaque would be to tip the balance of these "opposing forces" from T_h1, MI to an anti-inflammatory T_h2, M2 plaque milieu. Increased T_h2 cells have been linked in decreased risk of MI and stroke in women [69]. The majority of studies focusing on T_h2 cytokines consider them to be indirectly anti-atherogenic by dampening adaptive immunity, with subsequent lesion formation [70,71]. Additional studies exploring the potential protective effects of T_h2 interleukins on resident vascular cells such as ECs and VSMCs would also aid in identifying possible therapeutic targets.

Interleukin-4 (IL-4) is an anti-inflammatory cytokine that is confirmed to polarize T cells to their T_h2 phenotype [72]. Expression of IL-4 both at the mRNA and protein level has been confirmed in human atherosclerotic plaque [73]. IL-4 initiates a positive feedback loop as subsequent IL-4 induced T_h2 cells produce additional IL-4, which can further potentiate anti-inflammatory effects upon T cells and macrophages [47,74]. IL-4 polarizes T cells to T_h2 by inducing expression of the transcription factor, GATA-binding protein 3 (GATA3) and inhibiting expression of pro-inflammatory IFNγ [75]. Other *in vitro* experiments demonstrate anti-inflammatory phenotypes including decreased monocyte/macrophage adhesion and decreased VSMC proliferation. The role of IL-4 in atherogenesis in mouse models is controversial, as several studies present conflicting experimental data. Despite being an anti-inflammatory, T_h2 cytokine, IL-4 can induce expression of CAMs in ECs, resulting in increased immune cell extravasation into the vasculature, promoting inflammation. *Il4*$^{-/-}$x*Apoe*$^{-/-}$ double knockout mice have decreased plaque formation compared to controls [73]. Bone marrow transplants from *Il4*$^{-/-}$ into *Ldlr*$^{-/-}$ mice also results in decreased atherosclerosis. Additionally, treatment with IL-4 in *Apoe*$^{-/-}$ mice does not ameliorate plaque burden [76]. Despite conflicting data, some reviews still report IL-4 as an atheroprotective cytokine. The case of IL-4 highlights the complexity of inflammatory vascular disease. An anti-inflammatory cytokine does not always have a clear-cut role in disease pathology.

Interleukin-10 (IL-10) is the archetypal anti-inflammatory cytokine that plays a key role in regulating T_h1/T_h2 and M1/M2 balance, pushing toward T_h2 and M2 [77]. IL-10 has been extensively researched in atherosclerosis and has been established as an anti-atherosclerotic cytokine with multiple mechanisms in addition to T_h2 and M2 polarization. IL-10 is able to dampen expression of inflammatory genes in various cell types and inhibit antigen presentation and T cell proliferation [47]. Ongoing studies continue to explore the complex molecular mechanisms of IL-10 mediated atheroprotection. IL-10 signals through the IL-10 receptor and subsequent Jak1 and STAT 1, 3, and 5, though primarily STAT3 activation. IL-10 is able to block signaling cascades induced by master pro-inflammatory switches TNFα and NFκB in macrophages, ECs, and VSMCs. IL-10 is also able to dampen expression of pro-inflammatory transcripts by decreasing levels of the RNA binding protein (RBP), human antigen R (HuR) [78]. HuR stabilizes pro-inflammatory transcripts by binding "AU" rich regulatory elements, termed AREs, in their 3′untranscribed regions (3′UTR). IL-10 mediated atheroprotection is considered to be immunomodulatory, as ECs and VSMCs do not express IL-10 [79]. *Il10*$^{-/-}$x*Apoe*$^{-/-}$ double knockout mice have significantly increased plaque burden as well an increased VSMC and inflammatory cell infiltration [80]. Similarly, *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice administered AAV2 overexpressing IL-10 and transplant of bone marrow from IL-10 transgenic mice into *Ldlr*$^{-/-}$ mice all demonstrate decreased lesion sizes compared to controls [81,82]. Taken together, several studies indicate that IL-10 is a potent immune modulator that reduces atherosclerosis at multiple levels, including gene expression, leukocyte extravasation, and polarization of adaptive immunity to the T_h2 phenotype.

Interleukin-19 (IL-19) is an anti-inflammatory T_h2 cytokine that is a member of the IL-10 family [83]. IL-19 signals through dimerization of the α- and β - subunits of the IL-20 receptor. IL-19 is expressed in macrophages and T cells, and unlike IL-10, can be induced in ECs and VSMCs by pro-inflammatory signals [84,85]. IL-19 is able to polarize T cells and macrophages to T_h2 and M2 phenotypes, promoting its role as anti-inflammatory [86,87]. IL-19 is also able to decrease expression of CAMs and reduce leukocyte/EC interactions [88]. Immunohistochemical analysis of human coronary arteries with class 4 plaques show increased IL-19 expression compared to healthy controls, suggesting IL-19 may play a compensatory role in atherogenesis [86]. IL-19 treatment decreases oxLDL lipid uptake in wild-type (WT) macrophages. Furthermore, *Il19*$^{-/-}$ macrophages demonstrate increased oxLDL lipid uptake compared to WT macrophages, suggesting that IL-19 may participate in reverse cholesterol transport, an emerging anti-atherosclerotic mechanism [87]. IL-19 is also able to influence activation of cultured VSMCs, reducing their proliferation, migration, and inflammatory gene expression [84,89,90]. *Ldlr*$^{-/-}$ mice treated with IL-19 while simultaneously being fed high fat diet have decreased plaque burden compared to PBS injected controls [86]. Interestingly, IL-19 does not affect NF-κB; its strong anti-inflammatory effects are hypothesized to be through inhibition of pro-inflammatory cytokine mRNA stability mediated by the RBP HuR [89]. A potential mechanism is that IL-19 prevents HuR translocation from the nucleus to the cytoplasm, thereby blocking its ability to bind and stabilize pro-inflammatory transcripts. The emerging picture for IL-19 shows potent anti-atherosclerotic effects through its ability to engage multiple cell types and affect multiple mechanisms including reduction in inflammatory gene expression, macrophage polarization, reduction in extravasation, and increased reverse cholesterol transport.

Interleukin-33 (IL-33) is a member of the IL-1 family and is normally expressed in the healthy vasculature by multiple cell types [91]. It is able to polarize to T_h2 and M2 by induction of IL-4, IL-5 and IL-13 and by decreasing expression of IFNγ [92]. IL-33 expression is increased in human atherosclerotic plaque, specifically in macrophages. IL-33 has been shown to reduce expression of scavenger receptors and enhance expression of proteins involved in cholesterol efflux, thereby reducing foam cell formation [93]. IL-33 treatment in *Apoe*$^{-/-}$ mice has resulted in decreased atherosclerosis as well as reduced macrophage infiltration in atherosclerotic plaques, though these data were from two independent studies and have not yet both been assessed together [91].

Transforming growth factor beta (TGF β) is an anti-atherosclerotic, T_h2 cytokine that is part of the TGF super-family. TGFβ is the most investigated cytokine in the TGF family and is able to negatively regulate pro-inflammatory signaling. Global *Tgfβ*$^{-/-}$ mice are postnatally lethal with significant leukocyte infiltration in all organs, suggesting a vital immunoregulatory role. TGFβ is present in human atherosclerotic plaques and can be secreted by T cells, macrophages, ECs, and VSMCs. TGFβ has a number of effects including influencing T cell

differentiation and immune cell modulation [47]. TGFβ is a known pro-fibrotic and wound-healing cytokine, and initial studies exploring the role of TGFβ in atherosclerosis focused on its effects on VSMCs. Importantly, TGFβ contributes to matrix deposition, which is crucial for maintaining plaque stability in humans. Abrogation of TGFβ in T cells results in exacerbated lesion sizes with decreased VSMCs and collagen in *Apoe*$^{-/-}$ mice. Matrix deposition is considered a stability characteristic and thus, this suggests a potential role for TGFβ in promotion of plaque stability in humans [94]. Additionally, global inhibition of TGFβ accelerates plaque deposition and also decreases collagen content, further implicating a protective role in plaque vulnerability in humans [95]. Patients with severe cases of atherosclerosis have decreased levels of circulating TGFβ in their sera, providing further clinical evidence for TGFβ as an anti-atherosclerotic cytokine [96].

5. Post-transcriptional control of cytokine expression

Cytokine expression is induced by numerous factors present within the plaque milieu. Many excellent reviews have described transcriptional activation of cytokine expression but fewer describe mechanisms involved in post-transcriptional modifications that regulate cytokine expression, particularly cytokine mRNA stability [97–99]. A major point of control for the cell to regulate cytokine abundance is regulation of cytokine mRNA stability. This section will describe what is known about RNA binding proteins (RBPs) that regulate cytokine abundance by modulation of mRNA. Although modulation of mRNA has been posited as a possible therapeutic strategy, surprisingly, there is very little literature exploring the concept that it could be directly regulated by inflammatory stimuli. In this regard, regulation of cytokine mRNA stability can be considered an overlooked therapeutic opportunity [100]. To this end, RBPs have emerged as critical regulators of cytokine expression and potential targets of drug therapy. RBPs appear to play a major role in phenotypic switching of several cell types of vascular disease. While RNA stability will be predominantly discussed, it is important to note that in addition to influencing stability, RBPs are also able to alter mRNA composition and localization, and mediate translation.

Many cytokines have unstable mRNAs, often due to the presence of *cis*-acting AU-rich elements (AREs) in the 3′UTR, which promote degradation. Trans-acting factors, including RBPs, are able to bind the AREs and regulate mRNA fate. AREs were first identified in 1986 and subsequent studies established them as a mechanism of decay of inflammation related transcripts [101]. It is now known that RBPs are able to bind AREs and either stabilize or destabilize them, leading to translation or degradation respectively. Based on a database of human mRNAs, roughly 8% contain AREs, but the percentage of cytokines that contain them is much higher, attributing to their tightly controlled expression. Controlling mRNA decay allows the cell to fine-tune mRNA abundance and translation for a quick adaptation to inflammatory conditions. It has been demonstrated that cytokines expressed early in inflammation have several AREs compared to those expressed later. Multiple RBPs can bind the same transcript and influence protein levels by different mechanisms.

TNFα is an example of a potent, rapid-response pro-inflammatory cytokine whose mRNA is finely controlled by RBPs, allowing the cell to tightly regulate induction of other cytokines. TNFα is known to initiate and sustain pathogenesis of a number of inflammatory diseases including atherosclerosis, due to its central role in inflammation. TNFα is perhaps the most studied cytokine in terms of mechanisms of regulation. The TNFα 3′UTR is complex and offers the greatest opportunity to identify and understand RBPs. We will use TNFα as an example of how the 3′UTR of mRNA can be used by RBPs to regulate cytokine abundance. TNFα mRNA contains multiple class II AREs in the 3′UTR, which are overlapping copies of nonamer UUAUUUAUU within the AU-rich region. Post-transcriptional control of TNFα by its ARE occurs at multiple points. The TNFα AREs are generally targeted to destabilize its transcripts and initiate mRNA decay, though at least one RBP that promotes stability has been identified. Spontaneous mouse models of autoimmune disease have decreased levels of TNFα that are associated with mutations in their TNFα ARE sequences [102]. Genetic deletion of TNFα AREs in mice (TNF$^{\Delta ARE}$) results in chronic TNFα protein overproduction, demonstrating the importance of these elements in regulation of TNFα abundance [103]. TNF$^{\Delta ARE}$ mice also develop chronic inflammatory arthritis and inflammatory bowel disease. Unstimulated thioglycollate-elicited peritoneal macrophages (TEPM) and bone marrow derived macrophages (BMDM) isolated from TNF$^{\Delta ARE}$ mice spontaneously produce detectable levels of TNFα protein, and when stimulated with LPS exhibit a 3- to 5- fold increase in TNFα protein compared to controls. When stimulated with LPS, steady state levels of TNFα mRNA from TNF$^{\Delta ARE}$ TEPM demonstrate a sustained, 148-fold increase over WT controls. Altogether, these data suggest the absence of AREs augments TNFα mRNA and subsequent protein abundance, and demonstrate the importance of these elements in regulation of cytokine levels. Effects of deletion of AREs in TNFα in the scope of atherosclerosis and other vascular diseases have yet to be investigated. Several ARE-binding RBPs that influence TNFα have been identified. The following section will briefly describe the function of each and its role in regulation of TNFα mRNA (See Table 2).

Human antigen R (HuR) is a ubiquitously expressed member of the embryonic lethal abnormal vision (ELAV) family of RBPs. HuR activity is linked to its translocation from the nucleus to the cytoplasm where mRNA is processed. HuR has been shown to recognize and bind AREs in the 3′UTR of many cytokine transcripts [104]. *Hur*$^{-/-}$ mice are embryonically lethal, demonstrating its importance in mRNA processing. Initial studies exploring HuR in RNA stability found HuR to bind unstable transcripts and suggested HuR was a destabilizing RBP. However, follow up *in vitro* studies in which HuR was silenced, vascular endothelial growth factor (VEGF) mRNA, which contains an ARE in its 3′UTR, was destabilized [105]. Furthermore, VEGF transcripts were stabilized in cells when HuR was overexpressed. Experiments performed in an LPS-sensitive macrophage line to identify proteins that bind AREs in TNFα recognized HuR as the RBP with the highest affinity. Overexpression of HuR in Tet-OFF HeLa cells stabilized reporter construct containing the human TNFα ARE [106]. HuR appears to be the only identified RBP confirmed to stabilize TNFα transcripts. It is likely that there is competition among RBPs to bind AREs and influence protein production. HuR and the RBP TIA-1 are known to cooperatively bind TNFα, which results in destabilization [104]. IL-10 and IL-19 have both been shown to dampen inflammation by decreasing HuR abundance and/or activity, resulting in destabilization of pro-inflammatory transcripts including TNFα. Though not yet reported, HuR activity would likely be a promising target in anti-atherosclerotic therapy.

Tristetraprolin (TTP) is a Cys-Cys-Cys-His (CCCH) zinc finger protein. In contrast to HuR, TTP destabilizes TNFα transcripts by binding its AREs. *Ttp*$^{-/-}$ bone marrow and fetal liver derived macrophages exhibit increased TNFα mRNA and protein in response to LPS *in vitro*. *Ttp*$^{-/-}$ mice begin developing arthritis, dermatitis, autoimmunity, and myeloid hyperplasia shortly after birth. Virtually all these syndromes can be reversed by treatment with TNFα antibody, suggesting these phenotypes are due to uncontrolled TNFα expression. *Ttp*$^{-/-}$ mice also display increased TNFα at the mRNA and protein levels both with and without LPS stimulation [107]. Phosphorylation of TTP results in its sequestration and subsequent increased TNFα production as TTP can no longer bind the transcript.

ARE/poly(U)-binding/degradation factor (AUF-1) is also known as heterogeneous nuclear ribonucleoprotein D0 (HNRNP D). AUF-1 is ubiquitously expressed and is able to bind poly-(U) sites on mRNAs, in addition to AREs [108]. Knockdown of AUF-1 in WT BMDM results in delayed degradation of TNFα transcripts, suggesting that like TTP, AUF-1 is an mRNA destabilization factor. *Auf*$^{-/-}$ mice have increased sensitivity to LPS-induced sepsis and also develop endotoxemia and chronic dermatitis with increased mortality and chronic systemic

Table 2
RNA binding proteins and predicted effects on atherogenicity.

RNA-binding protein	Abbreviation	Effect on TNFα mRNA levels	Mechanism	Predicted atherogenicity
Human antigen R	HuR	Increase [105,106]	Stabilizes RNA [105,106]	Pro-atherogenic
Tristetraprolin	TTP	Decrease [107]	Destabilizes RNA [107]	Anti-atherogenic
ARE/poly(U)-binding/degradation factor	AUF-1	Decrease [108]	Destabilizes RNA [108]	Anti-atherogenic
T-cell-restricted intracellular antigen-1	TIA-1	Decrease [109]	Inhibits translation [110]	Anti-atherogenic
TIA-1-related protein	TIAR	Decrease [109]	Inhibits translation [110]	Anti-atherogenic
CUG triplet repeat RNA binding protein 1	CUGBP1	Decrease [113]	Destabilizes RNA [113,114]	Anti-atherogenic

inflammation due to failure to effectively degrade TNFα, as well as IL-1β transcripts. Experiments completed in transgenic mice have confirmed destabilization of TNFα, but interestingly have found stabilization of other ARE-containing transcripts. It has been suggested that AUF-1 has different effects on mRNAs depending on cell type. This is likely through interaction of AUF-1 with other cell type specific factors that modulate its activity, providing an additional level of control of cytokine abundance.

T-cell-restricted intracellular antigen-1 (TIA-1) and TIA-1-related protein (TIAR) are both members of the RNA-recognition motif family of RBPs and have both been shown to bind AREs on TNFα transcripts. TIA-1 and TIAR are able to repress TNFα protein production by blocking translation. Peritoneal macrophages isolated from *Tia1*$^{-/-}$ mice express the same amount of TNFα mRNA as macrophages isolated from WT mice, however *Tia1*$^{-/-}$ macrophages produce excess TNFα protein compared to WT macrophages [109]. TNFα mRNA in *Tia1*$^{-/-}$ and WT macrophages do not differ in half-life, suggesting TIA-1 does not play a role in the stability of the transcript. Instead, TIA-1 and TIAF specifically silence translation [110]. Under stressed conditions TIA-1 and TIAR are able to prevent initiation of translation by recruiting and sequestering mRNAs to discrete cytoplasmic foci called stress granules. The role of TIAR in regulation of TNFα mRNA has not been as established as TIA-1 because *Tiar*$^{-/-}$ mice are embryonically lethal. The only identified functionally distinct feature of TIA-1 and TIAR at this point is their interactions with other RBPs.

CUG triplet repeat RNA binding protein 1 (CUGBP1) was originally found to bind CUG repeats in the 3′UTR of mRNAs in myotonic dystrophy [111]. CUGBP has been shown to affect alternative splicing, modulation of translation of mRNAs, and deadenylation or poly(A) shortening [112]. CUGBP1 is able to destabilize TNFα mRNA and is able to bind its 3′UTR at multiple sites [113]. CUGBP1 is able to bind a region called embryonic deadenylation elements (EDENs) found in the 3′UTR, in addition to AREs. EDENs are typically found in proximity to AREs and data suggests that neighboring AREs are involved in EDEN-dependent deadenylation [114]. Thus, CUGBP is able to destabilize TNFα by binding both AREs and EDENs, leading to deadenylation and mRNA decay; though data suggests CUGBP binds to AREs with greater affinity than EDENs. Knockdown of CUGBP *in vitro* results in stabilization and subsequent increased abundance of TNFα transcripts [113]

RBPs have yet to be extensively researched within the specific scope of vascular disease. The few studies that have investigated RBPs in vascular disease suggest an important role in phenotypic switching [115]. ARE-binding RBPs, as well as RBPs Quaking (QKI) and Roquin are able to influence the development of foam cells from monocytes and macrophages [116–118]. QKI is highly abundant in macrophages in advanced atherosclerotic lesions and abrogation of QKI is able to prevent monocyte extravasation and foam cell formation both *in vitro* and *in vivo* [118]. RNA-seq and microarray analysis suggest a role for QKI in mRNA abundance and alternative splicing of transcripts. One study has shown that Roquin is able to initiate TNFα degradation in macrophages by binding constitutive decay elements in its 3′UTR [116]. Additionally, HuR and QKI are able to affect dedifferentiation of VSMCs from a contractile to synthetic state [119,120]. Stimulation of human VSMCs with platelet-derived growth factor results in greater levels of cytoplasmic HuR and subsequent changes in expression of genes influencing cell proliferation, structure, and metabolism and knockdown of HuR is able to reduce VSMC proliferation [120]. QKI is required for development of the vasculature and QKI global knockout mice are embryonic lethal [121]. One recently identified mechanism by which QKI is able to influence VSMCs phenotype is by influencing alternative splicing of Myocardin pre-RNA, resulting in an imbalance of splice variants [119]. Additional studies are necessary to determine the direct role QKI and other RBPs play in development of atherosclerosis. Based on our current understanding of their function, we can predict that those that stabilize ARE-containing transcripts would be pro-atherosclerotic, while those that destabilize them would be atheroprotective (See Table 2).

6. Summary and conclusions

Vascular disease and atherosclerosis in particular, is and will continue to be a significant socioeconomic burden in the developed world. As a dynamic and complex interaction of many cell types in various stages of pro- and anti-inflammatory states, atherosclerosis initiation and progression relies on cytokines for communication across the various cell types. The balance between pro- and anti-inflammatory cytokines can potentially tip the balance between plaque progression and plaque regression. Post-transcriptional processes are a major point of regulation of cytokine synthesis, and several proteins participate in regulation of cytokine mRNA abundance. Understanding the mechanisms that regulate cytokine synthesis is key to our ability to identify therapeutic approaches to combat these significant diseases.

Acknowledgements

This work was supported by grants HL115575 and HL117724 from the National Heart Lung, and Blood Institute of the National Institutes of Health, and Grant 13GRNT1685003 from the American Heart Association to MVA. MR was supported by American Heart Association pre-doctoral fellowship 16PRE31220005.

References

[1] E.J. Benjamin, et al., Heart disease and stroke statistics-2017 update: a report from the American Heart Association, Circulation 135 (10) (2017) e146–e603.
[2] R. Ross, Atherosclerosis–an inflammatory disease, New Engl. J. Med. 340 (2) (1999) 115–126.
[3] G.M. Chisolm 3rd., Y. Chai, Regulation of cell growth by oxidized LDL, Free Radical Biol. Med. 28 (12) (2000) 1697–1707.
[4] I. Tabas, D.A. Weiland, A.R. Tall, Unmodified low density lipoprotein causes cholesteryl ester accumulation in J774 macrophages, Proc. Natl. Acad. Sci. USA 82 (2) (1985) 416–420.
[5] Q. Ye, et al., Inflammatory stress increases unmodified LDL uptake via LDL receptor: an alternative pathway for macrophage foam-cell formation, Inflamm. Res. 58 (11) (2009) 809–818.
[6] D.F. Ketelhuth, et al., Identification of a danger-associated peptide from apolipoprotein B100 (ApoBDS-1) that triggers innate proatherogenic responses, Circulation 124 (22) (2011) 2433–2443 1–7.
[7] A. Hermansson, et al., Immunotherapy with tolerogenic apolipoprotein B-100-loaded dendritic cells attenuates atherosclerosis in hypercholesterolemic mice, Circulation 123 (10) (2011) 1083–1091.
[8] A. Gistera, et al., Vaccination against T-cell epitopes of native ApoB100 reduces vascular inflammation and disease in a humanized mouse model of atherosclerosis, J. Intern. Med. 281 (4) (2017) 383–397.
[9] A. Assinger, et al., Apolipoprotein B100 danger-associated signal 1 (ApoBDS-1)

triggers platelet activation and boosts platelet-leukocyte proinflammatory responses, Thromb. Haemost. 112 (2) (2014) 332–341.
[10] C. Napoli, et al., Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions, J. Clin. Invest. 100 (11) (1997) 2680–2690.
[11] H.C. Stary, et al., A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. a report from the committee on vascular lesions of the council on arteriosclerosis, American Heart Association, Circulation 89 (5) (1994) 2462–2478.
[12] Y.V. Bobryshev, Monocyte recruitment and foam cell formation in atherosclerosis, Micron 37 (3) (2006) 208–222.
[13] R.P. Tracy, et al., T-helper type 1 bias in healthy people is associated with cytomegalovirus serology and atherosclerosis: the multi-ethnic study of atherosclerosis, J. Am. Heart Assoc. 2 (3) (2013) e000117.
[14] X. Zhou, S. Stemme, G.K. Hansson, Evidence for a local immune response in atherosclerosis. CD4+ T cells infiltrate lesions of apolipoprotein-E-deficient mice, Am. J. Pathol. 149 (2) (1996) 359–366.
[15] O.J. de Boer, et al., Low numbers of FOXP3 positive regulatory T cells are present in all developmental stages of human atherosclerotic lesions, PLoS One 2 (8) (2007) e779.
[16] H. Ait-Oufella, et al., Natural regulatory T cells control the development of atherosclerosis in mice, Nat. Med. 12 (2) (2006) 178–180.
[17] C. De Duve, The role of lysosomes in cellular pathology, Triangle 9 (6) (1970) 200–208.
[18] F.A. Verreck, et al., Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria, Proc. Natl. Acad. Sci. USA 101 (13) (2004) 4560–4565.
[19] B.B. Aggarwal, S.C. Gupta, J.H. Kim, Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey, Blood 119 (3) (2012) 651–665.
[20] T. Skoog, et al., Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men, Eur. Heart J. 23 (5) (2002) 376–383.
[21] K.J. Woollard, et al., Pathophysiological levels of soluble P-selectin mediate adhesion of leukocytes to the endothelium through Mac-1 activation, Circ. Res. 103 (10) (2008) 1128–1138.
[22] X. Zhou, G.K. Hansson, Detection of B cells and proinflammatory cytokines in atherosclerotic plaques of hypercholesterolaemic apolipoprotein E knockout mice, Scand. J. Immunol. 50 (1) (1999) 25–30.
[23] G.D. Kalliolias, L.B. Ivashkiv, TNF biology, pathogenic mechanisms and emerging therapeutic strategies, Nat. Rev. Rheumatol. 12 (1) (2016) 49–62.
[24] N. Parameswaran, S. Patial, Tumor necrosis factor-alpha signaling in macrophages, Crit. Rev. Eukar. Gene Expr. 20 (2) (2010) 87–103.
[25] H.Y. Hsu, A.C. Nicholson, D.P. Hajjar, Inhibition of macrophage scavenger receptor activity by tumor necrosis factor-alpha is transcriptionally and post-transcriptionally regulated, J. Biol. Chem. 271 (13) (1996) 7767–7773.
[26] H.Y. Hsu, Y.C. Twu, Tumor necrosis factor-alpha -mediated protein kinases in regulation of scavenger receptor and foam cell formation on macrophage, J. Biol. Chem. 275 (52) (2000) 41035–41048.
[27] Z.S. Galis, et al., Enhanced expression of vascular matrix metalloproteinases induced in vitro by cytokines and in regions of human atherosclerotic lesions, Ann. NY Acad. Sci. 748 (1995) 501–507.
[28] M.B. Taubman, et al., Tissue factor in the pathogenesis of atherosclerosis, Thromb. Haemost. 78 (1) (1997) 200–204.
[29] L. Branen, et al., Inhibition of tumor necrosis factor-alpha reduces atherosclerosis in apolipoprotein E knockout mice, Arterioscler. Thromb. Vasc. Biol. 24 (11) (2004) 2137–2142.
[30] M. Canault, et al., Exclusive expression of transmembrane TNF-alpha in mice reduces the inflammatory response in early lipid lesions of aortic sinus, Atherosclerosis 172 (2) (2004) 211–218.
[31] P.C. Taylor, M. Feldmann, Anti-TNF biologic agents: still the therapy of choice for rheumatoid arthritis, Nat. Rev. Rheumatol. 5 (10) (2009) 578–582.
[32] L.T. Jacobsson, et al., Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis, J. Rheumatol. 32 (7) (2005) 1213–1218.
[33] W.G. Dixon, et al., Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor alpha therapy: results from the British Society for Rheumatology Biologics Register, Arthritis Rheumatol. 56 (9) (2007) 2905–2912.
[34] X. Wang, et al., Interleukin-1 beta induces expression of adhesion molecules in human vascular smooth muscle cells and enhances adhesion of leukocytes to smooth muscle cells, Atherosclerosis 115 (1) (1995) 89–98.
[35] J.J. Kaden, et al., Interleukin-1 beta promotes matrix metalloproteinase expression and cell proliferation in calcific aortic valve stenosis, Atherosclerosis 170 (2) (2003) 205–211.
[36] S.Y. Eun, et al., IL-1beta enhances vascular smooth muscle cell proliferation and migration via P2Y2 receptor-mediated RAGE expression and HMGB1 release, Vasc. Pharmacol. 72 (2015) 108–117.
[37] R. Elhage, et al., Differential effects of interleukin-1 receptor antagonist and tumor necrosis factor binding protein on fatty-streak formation in apolipoprotein E-deficient mice, Circulation 97 (3) (1998) 242–244.
[38] Y. Kamari, et al., Differential role and tissue specificity of interleukin-1alpha gene expression in atherogenesis and lipid metabolism, Atherosclerosis 195 (1) (2007) 31–38.
[39] T.F. Gajewski, J. Joyce, F.W. Fitch, Antiproliferative effect of IFN-gamma in immune regulation. III. Differential selection of TH1 and TH2 murine helper T lymphocyte clones using recombinant IL-2 and recombinant IFN-gamma, J. Immunol. 143 (1) (1989) 15–22.
[40] S. Upadhya, et al., Atherogenic effect of interleukin-2 and antiatherogenic effect of interleukin-2 antibody in apo-E-deficient mice, Angiology 55 (3) (2004) 289–294.
[41] T. Dietrich, et al., Local delivery of IL-2 reduces atherosclerosis via expansion of regulatory T cells, Atherosclerosis 220 (2) (2012) 329–336.
[42] T. Korn, et al., IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells, Proc. Natl. Acad. Sci. USA 105 (47) (2008) 18460–18465.
[43] X. Cheng, et al., The Th17/Treg imbalance in patients with acute coronary syndrome, Clin. Immunol. 127 (1) (2008) 89–97.
[44] M. Romano, et al., Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment, Immunity 6 (3) (1997) 315–325.
[45] S.A. Huber, et al., Interleukin-6 exacerbates early atherosclerosis in mice, Arterioscler. Thromb. Vasc. Biol. 19 (10) (1999) 2364–2367.
[46] B. Schieffer, et al., Impact of interleukin-6 on plaque development and morphology in experimental atherosclerosis, Circulation 110 (22) (2004) 3493–3500.
[47] K. Tse, et al., T cells in atherosclerosis, Int. Immunol. 25 (11) (2013) 615–622.
[48] C. Buono, et al., T-bet deficiency reduces atherosclerosis and alters plaque antigen-specific immune responses, Proc. Natl. Acad. Sci. USA 102 (5) (2005) 1596–1601.
[49] T.S. Lee, et al., The role of interleukin 12 in the development of atherosclerosis in ApoE-deficient mice, Arterioscler. Thromb. Vasc. Biol. 19 (3) (1999) 734–742.
[50] A.D. Hauer, et al., Blockade of interleukin-12 function by protein vaccination attenuates atherosclerosis, Circulation 112 (7) (2005) 1054–1062.
[51] T. Yoshimoto, et al., IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-gamma production, J. Immunol. 161 (7) (1998) 3400–3407.
[52] S. Blankenberg, et al., Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina, Circulation 106 (1) (2002) 24–30.
[53] R. Elhage, et al., Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice, Cardiovasc. Res. 59 (1) (2003) 234–240.
[54] Z. Mallat, et al., Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability, Circ. Res. 89 (7) (2001) E41–E45.
[55] S.C. Whitman, P. Ravisankar, A. Daugherty, Interleukin-18 enhances atherosclerosis in apolipoprotein E(-/-) mice through release of interferon-gamma, Circ. Res. 90 (2) (2002) E34–E38.
[56] F. Cheng, et al., Decreased plasma IL22 levels, but not increased IL17 and IL23 levels, correlate with disease activity in patients with systemic lupus erythematosus, Ann. Rheum. Dis. 68 (4) (2009) 604–606.
[57] H. Ikeuchi, et al., Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine, Arthritis Rheumatol. 52 (4) (2005) 1037–1046.
[58] Q. Xia, et al., Characterisation of IL-22 and interferon-gamma-inducible chemokines in human carotid plaque, Int. J. Cardiol. 154 (2) (2012) 187–189.
[59] S. Rattik, et al., IL-22 affects smooth muscle cell phenotype and plaque formation in apolipoprotein E knockout mice, Atherosclerosis 242 (2) (2015) 506–514.
[60] E. Duvallet, et al., Interleukin-23: a key cytokine in inflammatory diseases, Ann. Med. 43 (7) (2011) 503–511.
[61] A. Abbas, et al., Interleukin 23 levels are increased in carotid atherosclerosis: possible role for the interleukin 23/interleukin 17 axis, Stroke 46 (3) (2015) 793–799.
[62] J.E. McLaren, D.P. Ramji, Interferon gamma: a master regulator of atherosclerosis, Cytokine Growth Factor Rev. 20 (2) (2009) 125–135.
[63] Z. Mallat, H. Ait-Oufella, A. Tedgui, Regulatory T cell responses: potential role in the control of atherosclerosis, Curr. Opin. Lipidol. 16 (5) (2005) 518–524.
[64] M.L. Leon, S.H. Zuckerman, Gamma interferon: a central mediator in atherosclerosis, Inflamm. Res. 54 (10) (2005) 395–411.
[65] S. Gupta, et al., IFN-gamma potentiates atherosclerosis in ApoE knock-out mice, J. Clin. Invest. 99 (11) (1997) 2752–2761.
[66] S.C. Whitman, et al., Exogenous interferon-gamma enhances atherosclerosis in apolipoprotein E-/- mice, Am. J. Pathol. 157 (6) (2000) 1819–1824.
[67] S. Schulte, G.K. Sukhova, P. Libby, Genetically programmed biases in Th1 and Th2 immune responses modulate atherogenesis, Am. J. Pathol. 172 (6) (2008) 1500–1508.
[68] J. Frostegard, et al., Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines, Atherosclerosis 145 (1) (1999) 33–43.
[69] D. Engelbertsen, et al., T-helper 2 immunity is associated with reduced risk of myocardial infarction and stroke, Arterioscler. Thromb. Vasc. Biol. 33 (3) (2013) 637–644.
[70] J.H. von der Thusen, et al., Interleukins in atherosclerosis: molecular pathways and therapeutic potential, Pharmacol. Rev. 55 (1) (2003) 133–166.
[71] Z. Mallat, et al., Protective role of interleukin-10 in atherosclerosis, Circ. Res. 85 (8) (1999) e17–e24.
[72] S.L. Swain, et al., IL-4 directs the development of Th2-like helper effectors, J. Immunol. 145 (11) (1990) 3796–3806.
[73] P. Davenport, P.G. Tipping, The role of interleukin-4 and interleukin-12 in the progression of atherosclerosis in apolipoprotein E-deficient mice, Am. J. Pathol. 163 (3) (2003) 1117–1125.
[74] K.J. Moore, F.J. Sheedy, E.A. Fisher, Macrophages in atherosclerosis: a dynamic balance, Nat. Rev. Immunol. 13 (10) (2013) 709–721.
[75] O. Wurtz, M. Bajenoff, S. Guerder, IL-4-mediated inhibition of IFN-gamma production by CD4+ T cells proceeds by several developmentally regulated mechanisms, Int. Immunol. 16 (3) (2004) 501–508.
[76] V.L. King, L.A. Cassis, A. Daugherty, Interleukin-4 does not influence development

of hypercholesterolemia or angiotensin II-induced atherosclerotic lesions in mice, Am. J. Pathol. 171 (6) (2007) 2040–2047.
[77] F.O. Martinez, et al., Macrophage activation and polarization, Front. Biosci. 13 (2008) 453–461.
[78] P. Krishnamurthy, et al., IL-10 inhibits inflammation and attenuates left ventricular remodeling after myocardial infarction via activation of STAT3 and suppression of HuR, Circ. Res. 104 (2) (2009) e9–e18.
[79] J.H. Von Der Thusen, et al., Attenuation of atherogenesis by systemic and local adenovirus-mediated gene transfer of interleukin-10 in LDLr-/- mice, FASEB J. 15 (14) (2001) 2730–2732.
[80] G. Caligiuri, et al., Interleukin-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins in apolipoprotein E knockout mice, Mol. Med. 9 (1–2) (2003) 10–17.
[81] Y. Liu, et al., Inhibition of atherogenesis in LDLR knockout mice by systemic delivery of adeno-associated virus type 2-hIL-10, Atherosclerosis 188 (1) (2006) 19–27.
[82] T. Yoshioka, et al., Adeno-associated virus vector-mediated interleukin-10 gene transfer inhibits atherosclerosis in apolipoprotein E-deficient mice, Gene Ther. 11 (24) (2004) 1772–1779.
[83] S.C. Liao, et al., IL-19 induced Th2 cytokines and was up-regulated in asthma patients, J. Immunol. 173 (11) (2004) 6712–6718.
[84] Y. Tian, et al., Expression and suppressive effects of interleukin-19 on vascular smooth muscle cell pathophysiology and development of intimal hyperplasia, Am. J. Pathol. 173 (3) (2008) 901–909.
[85] S. Jain, et al., The anti-inflammatory cytokine interleukin 19 is expressed by and angiogenic for human endothelial cells, Arterioscler. Thromb. Vasc. Biol. 31 (1) (2011) 167–175.
[86] S. Ellison, et al., Attenuation of experimental atherosclerosis by interleukin-19, Arterioscler. Thromb. Vasc. Biol. 33 (10) (2013) 2316–2324.
[87] K. Gabunia, et al., IL-19 halts progression of atherosclerotic plaque, polarizes, and increases cholesterol uptake and efflux in macrophages, Am. J. Pathol. 186 (5) (2016) 1361–1374.
[88] R.N. England, et al., Interleukin-19 decreases leukocyte-endothelial cell interactions by reduction in endothelial cell adhesion molecule mRNA stability, Am. J. Physiol. Cell Physiol. 305 (3) (2013) C255–C265.
[89] A.A. Cuneo, D. Herrick, M.V. Autieri, Il-19 reduces VSMC activation by regulation of mRNA regulatory factor HuR and reduction of mRNA stability, J. Mol. Cell Cardiol. 49 (4) (2010) 647–654.
[90] K. Gabunia, et al., Anti-inflammatory cytokine interleukin-19 inhibits smooth muscle cell migration and activation of cytoskeletal regulators of VSMC motility, Am. J. Physiol. Cell Physiol. 300 (4) (2011) C896–C906.
[91] A.M. Miller, et al., IL-33 reduces the development of atherosclerosis, J. Exp. Med. 205 (2) (2008) 339–346.
[92] F.Y. Liew, J.P. Girard, H.R. Turnquist, Interleukin-33 in health and disease, Nat. Rev. Immunol. 16 (11) (2016) 676–689.
[93] J.E. McLaren, et al., IL-33 reduces macrophage foam cell formation, J. Immunol. 185 (2) (2010) 1222–1229.
[94] A. Gojova, et al., Specific abrogation of transforming growth factor-beta signaling in T cells alters atherosclerotic lesion size and composition in mice, Blood 102 (12) (2003) 4052–4058.
[95] Z. Mallat, et al., Inhibition of transforming growth factor-beta signaling accelerates atherosclerosis and induces an unstable plaque phenotype in mice, Circ. Res. 89 (10) (2001) 930–934.
[96] D.J. Grainger, et al., The serum concentration of active transforming growth factor-beta is severely depressed in advanced atherosclerosis, Nat. Med. 1 (1) (1995) 74–79.
[97] T. Matsusaka, et al., Transcription factors NF-IL6 and NF-kappa B synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8, Proc. Natl. Acad. Sci. USA 90 (21) (1993) 10193–10197.
[98] J.N. Ihle, STATs: signal transducers and activators of transcription, Cell 84 (3) (1996) 331–334.
[99] A. Tedgui, Z. Mallat, Cytokines in atherosclerosis: pathogenic and regulatory pathways, Physiol. Rev. 86 (2) (2006) 515–581.
[100] W. Eberhardt, et al., Modulation of mRNA stability as a novel therapeutic approach, Pharmacol. Ther. 114 (1) (2007) 56–73.
[101] D. Caput, et al., Identification of a common nucleotide sequence in the 3′-untranslated region of mRNA molecules specifying inflammatory mediators, Proc. Natl. Acad. Sci. USA 83 (6) (1986) 1670–1674.
[102] C.O. Jacob, S.K. Lee, G. Strassmann, Mutational analysis of TNF-alpha gene reveals a regulatory role for the 3′-untranslated region in the genetic predisposition to lupus-like autoimmune disease, J. Immunol. 156 (8) (1996) 3043–3050.
[103] D. Kontoyiannis, et al., Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies, Immunity 10 (3) (1999) 387–398.
[104] V. Palanisamy, et al., Control of cytokine mRNA expression by RNA-binding proteins and microRNAs, J. Dent. Res. 91 (7) (2012) 651–658.
[105] N.S. Levy, et al., Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR, J. Biol. Chem. 273 (11) (1998) 6417–6423.
[106] J.L. Dean, et al., The 3′ untranslated region of tumor necrosis factor alpha mRNA is a target of the mRNA-stabilizing factor HuR, Mol. Cell. Biol. 21 (3) (2001) 721–730.
[107] G.A. Taylor, et al., A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency, Immunity 4 (5) (1996) 445–454.
[108] F.M. Gratacos, G. Brewer, The role of AUF1 in regulated mRNA decay, Wiley Interdiscip. Rev. RNA 1 (3) (2010) 457–473.
[109] M. Piecyk, et al., TIA-1 is a translational silencer that selectively regulates the expression of TNF-alpha, EMBO J. 19 (15) (2000) 4154–4163.
[110] N.L. Kedersha, et al., RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules, J. Cell. Biol. 147 (7) (1999) 1431–1442.
[111] L.T. Timchenko, et al., Identification of a (CUG)n triplet repeat RNA-binding protein and its expression in myotonic dystrophy, Nucleic Acids Res. 24 (22) (1996) 4407–4414.
[112] K.C. Moraes, C.J. Wilusz, J. Wilusz, CUG-BP binds to RNA substrates and recruits PARN deadenylase, RNA 12 (6) (2006) 1084–1091.
[113] L. Zhang, et al., The RNA-binding protein CUGBP1 regulates stability of tumor necrosis factor mRNA in muscle cells: implications for myotonic dystrophy, J .Biol. Chem. 283 (33) (2008) 22457–22463.
[114] Y. Audic, F. Omilli, H.B. Osborne, Embryo deadenylation element-dependent deadenylation is enhanced by a cis element containing AUU repeats, Mol. Cell. Biol. 18 (12) (1998) 6879–6884.
[115] R.G. de Bruin, et al., Emerging roles for RNA-binding proteins as effectors and regulators of cardiovascular disease, Eur. Heart J. 38 (18) (2017) 1380–1388.
[116] K. Leppek, et al., Roquin promotes constitutive mRNA decay via a conserved class of stem-loop recognition motifs, Cell 153 (4) (2013) 869–881.
[117] S. Sakurai, U. Ohto, T. Shimizu, Structure of human Roquin-2 and its complex with constitutive-decay element RNA, Acta Crystallogr. F Struct. Biol. Commun. 71 (Pt 8) (2015) 1048–1054.
[118] R.G. de Bruin, et al., Quaking promotes monocyte differentiation into pro-atherogenic macrophages by controlling pre-mRNA splicing and gene expression, Nat. Commun. 7 (2016) 10846.
[119] E.P. van der Veer, et al., Quaking, an RNA-binding protein, is a critical regulator of vascular smooth muscle cell phenotype, Circ. Res. 113 (9) (2013) 1065–1075.
[120] R. Pullmann Jret al., Enhanced proliferation of cultured human vascular smooth muscle cells linked to increased function of RNA-binding protein HuR, J. Biol. Chem. 280 (24) (2005) 22819–22826.
[121] J.K. Noveroske, et al., Quaking is essential for blood vessel development, Genesis 32 (3) (2002) 218–230.