

Cytokines and atherosclerosis: a comprehensive review of studies in mice

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In the past few years, inflammation has emerged as a major driving force of atherosclerotic lesion development. It is now well-established that from early lesion to vulnerable plaque formation, numerous cellular and molecular inflammatory components participate in the disease process. The most prominent cells that invade in evolving lesions are monocyte-derived macrophages and T-lymphocytes. Both cell types produce a wide array of soluble inflammatory mediators (cytokines, chemokines) which are critically important in the initiation and perpetuation of the disease. This review summarizes the currently available information from mouse studies on the contribution of a specified group of cytokines expressed in atherosclerotic lesions, viz. interleukins (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, IL-20) and macrophage-associated cytokines [tumour necrosis factor- α (TNF- α); macrophage migration inhibitory factor (MIF); interferon- γ (IFN- γ); colony stimulating factors G-CSF, M-CSF, GM-CSF) to atherogenesis. Emphasis is put on the consistency of the effects of these cytokines, i.e. inasmuch an effect depends on the experimental approach applied (overexpression/deletion, strain, gender, dietary conditions, and disease stage). An important outcome of this survey is (i) that only for a few cytokines there is sufficient *consistent* data allowing classifying them as typically proatherogenic (IL-1, IL-12, IL-18, MIF, IFN- γ , TNF- α , and M-CSF) or antiatherogenic (IL-10) and (ii) that some cytokines (IL-4, IL-6 and GM-CSF) can exert pro- or anti-atherogenic effects depending on the experimental conditions. This knowledge can be used for improved early detection, prevention and treatment of atherosclerosis.

1. Introduction

It is now well-accepted that atherosclerosis is not only merely a lipid disorder, but also a chronic inflammatory disease.¹ Inflammation is recognized as a major contributor to atherogenesis through adverse effects on lipoprotein metabolism and arterial wall biology, and both the innate (monocyte-derived macrophages) and the acquired immune system (T-cells) have been implicated in the atherogenic process.^{1,2} Monocytes and T-cells migrate from the circulation into the intima of the arterial wall where they differentiate into macrophages, which then take up modified lipoproteins thereby transforming into foam cells. Monocyte-derived macrophages are abundantly present at all stages of the disease process.^{1,3} Their pivotal role in atherogenesis has been demonstrated by the attenuation of

lesion formation in monocyte-deficiency in both ApoE^{-/-} mice and LDLR^{-/-} mice.^{4,5}

In addition to cells of the monocyte/macrophage lineage, CD4⁺ and CD8⁺ T-cells are present in atherosclerotic lesions. Macrophages and T-lymphocytes together produce a wide array of cytokines that can exert both pro- and anti-inflammatory effects (*Figure 1*). Pro-inflammatory cytokines of the interleukin category are considered to be key players in the chronic vascular inflammation that is typical for atherosclerosis.³ Presence of interleukins and their receptors has been demonstrated in atheromatous tissue, and the serum levels of several of these cytokines have been found to correlate positively with (coronary) arterial disease and its sequelae. Besides interleukins, macrophage-associated cytokines such as tumour necrosis factor (TNF)- α , macrophage migration inhibitory factor (MIF), interferon (IFN)- γ and colony stimulating factors (CSFs) have emerged as key factors in the pathogenesis of atherosclerosis.^{2,6}

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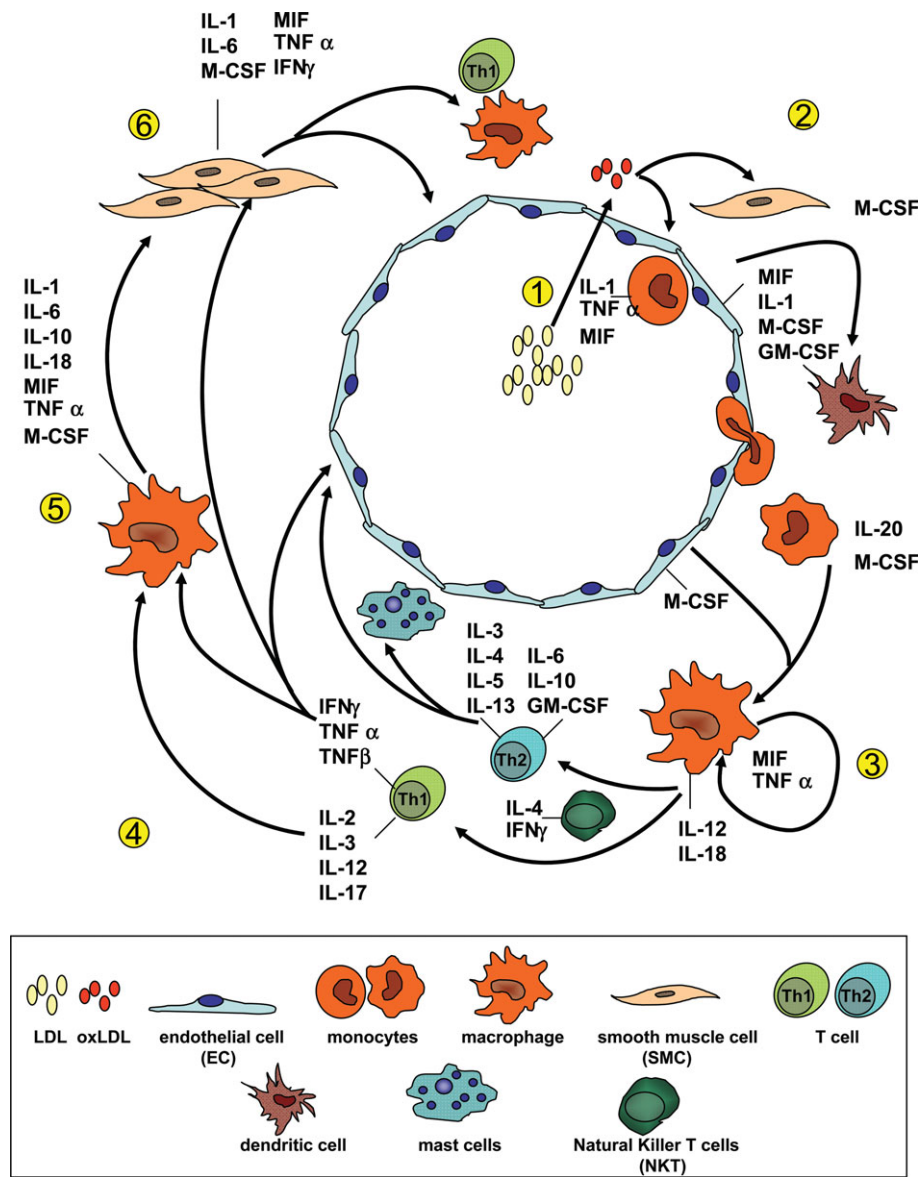


Figure 1 Cytokines involved in atherogenesis and their cellular source and targets. (1) LDL particles penetrate the endothelial cell layer and are oxidized in the intima (oxLDL). Pro-inflammatory lipids are released from oxLDL and induce expression of cytokines in EC (e.g. IL-1, MIF, M-CSF, and GM-CSF) and SMCs (M-CSF). MIF and M-CSF can function as a chemotactic factor for monocytes and T-cells. GM-CSF is a major regulator of dendritic cell differentiation and involved in lesional dendritic cell accumulation. (2) TNF- α and MIF are involved in the autocrine activation of macrophages thereby amplifying inflammation. IL-12 and IL-18 formed by macrophages and IL-4 (e.g. from NKT) promote the differentiation of native T-cells into T_H1 -cells and T_H2 -cells, respectively. IL-12 and IL-18 are potent inducers of IFN- γ . (3) T_H1 -cells can further activate macrophages (inflammatory cascade) whereas T_H2 -cells produce anti-inflammatory mediators (e.g. IL-4, IL-10, and IL-13) with opposite effect on macrophages, T-cells and EC. IL-4 (T_H2 -associated) impair the development of T_H1 -cells from pre- T_H -cells (not shown). Vice versa, IFN- γ inhibits T_H2 -cell development. Also, T_H2 -derived IL-4 and IL-13 are potent stimulators of antibody production (on B-cells, not shown), IL-5 is involved in B-cell differentiation and eosinophilic inflammation. (4) Macrophages are further stimulated by T-cell derived IFN- γ as well as IL-1 and TNF- α (also from other sources) together resulting in an amplification of the inflammatory response. (5) SMCs are targets for many of the macrophage- and T-cell-derived cytokines and participate in this self-perpetuating inflammatory cycle by producing and secreting a range of pro-inflammatory factors among which are IL-1, TNF- α , and IFN- γ .

More refined analysis of local vascular inflammation and the cytokines expressed in atherosclerotic plaques revealed that there is a balance between pro-inflammatory and anti-inflammatory cytokines and that this balance is crucial for lesion development (Figure 2A). For example, the secreted cytokines of type 1 CD4⁺ T-helper cells (T_H1 cells) such as interleukin-2 (IL-2), IL-12, IFN- γ , TNF- α and TNF- β are pro-inflammatory and exacerbate atherosclerotic disease, whereas T_H2 cytokines such as IL-4, IL-5, IL-10, and IL-13 are considered to be mainly atheroprotective and can counteract T_H1 cytokine activity/production.⁷⁻⁹ Notably,

this attractive black-and-white concept of T_H1 and T_H2 responses controlling the development of atherosclerosis may not be true at all stages of plaque development.¹⁰

Important insight into the role of cytokines in atherogenesis has been gained from animal studies, in particular mouse studies. Mouse atherosclerosis models resemble humans in that the most prominent cells involved in lesion development are monocyte-derived macrophages and T-lymphocytes. It is important to note, however, that all standard mouse atherosclerosis models (e.g. ApoE^{-/-} mice, LDLR^{-/-} mice, and ApoE*3Leiden-mice)¹¹ are on a C57/BL6 background

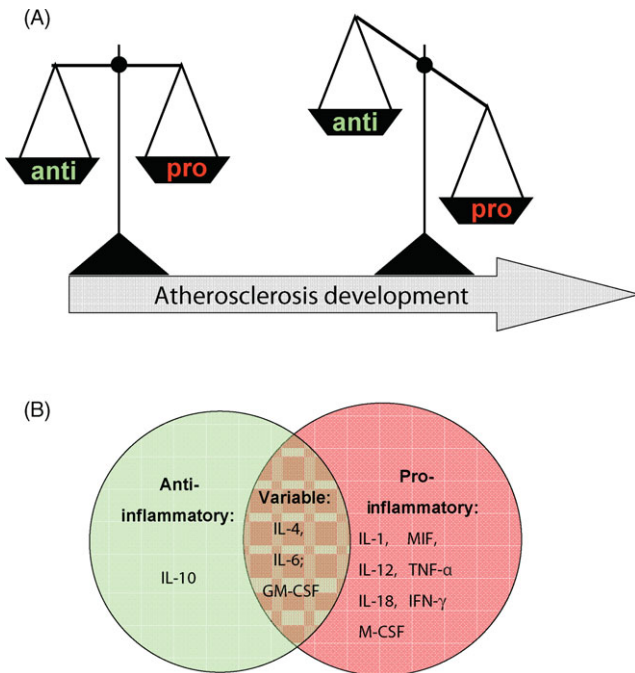


Figure 2 Categorization of cytokines into pro-atherogenic and anti-atherogenic cytokines based on their effects in mouse atherosclerosis models. (A) The balance between pro-inflammatory and anti-inflammatory cytokines is crucial for lesion development and imbalance is colloquially referred to exacerbate atherosclerotic disease. (B) Overview of the cytokines with consistent anti-atherogenic effects (green circle), pro-atherogenic effects (red circle), or variable, dual function (intersection).

which is prone to T_H1 -type immune responses and inherently underestimates the impact of T_H2 -type responses.

The T_H1 cytokines IL-1 β and TNF- α are among the most important cytokine mediators of the inflammatory response,¹² and concordantly a large number of studies have attempted to delineate the role of these cytokines in atherogenesis. However, the role of other members of the interleukin family in atherogenesis is increasingly appreciated. Here, we summarize all currently available information from mouse atherosclerosis studies investigating the role of the interleukins IL-1,-2,-4,-5,-6,-10,-12,-18, and -20, the macrophage-associated cytokines TNF- α , MIF, IFN- γ , and the colony stimulating factors G-CSF, M-CSF, and GM-CSF. Particular attention is paid to the experimental conditions employed (cf, strain, gender, type of diet, disease stage, and mode of intervention; experimental details are provided in *Table 1*) as different or even contradictory study outcomes may be related to differences in experimental set-up.

2. Interleukin-1

IL-1 is a prototypic pro-inflammatory cytokine that exists in two forms, IL-1 α and IL-1 β , both of which exert similar but not completely overlapping biological functions mediated through the IL-1 receptor (IL-1R). After binding to IL-1R, IL-1 induces the production of a broad spectrum of cytokines and chemokines as well as the expression of adhesion molecules on endothelial cells (EC), thus leading to the recruitment of inflammatory cells. In addition, IL-1 contributes to the development of vascular damage by stimulating cell proliferation and differentiation and the release of matrix-degrading enzymes. The IL-1R antagonist (IL-1Ra) is

a structural homologue of IL-1 that binds to IL-1R but does not induce any cellular responses and is therefore a natural inhibitor of IL-1-activity.¹³

The role of IL-1 in atherogenesis has been investigated through influencing its level or activity (*Table 1*). Elhage *et al.*¹⁴ showed that short-term perfusion with a recombinant IL-1Ra preparation reduces fatty-streak formation in male and female ApoE^{-/-}-mice fed a cholesterol/choleate-rich diet, without evident interference with lipid metabolism. This study lacked, however, proper controls (e.g. no sham minipump). Merhi-Soussi *et al.*¹⁵ demonstrated that atherosclerosis development is inhibited in ApoE^{-/-}-mice overexpressing either secreted IL-1Ra or intracellular IL-1Ra and fed a cholesterol-rich diet, thus providing more solid proof to the observations by Elhage.

Devlin *et al.*¹⁶ used two strains of mice, either with deficient or excess IL-1Ra. IL-1Ra^{-/-}/C57/BL6/J-mice fed a cholesterol/choleate-diet had a 3-fold decrease in non-HDL-cholesterol and a trend toward increased foam-cell lesion area compared to wild-type littermate controls. LDLR^{-/-}-mice expressing high levels of murine IL-1Ra and fed a cholesterol-saturated fat diet showed a 40% increase in non-HDL-cholesterol, consistent with the IL-1Ra^{-/-}-data, but there was no significant change in lesion size. However, when IL-1Ra-overexpressing LDLR^{-/-}-mice were fed a high-cholesterol(HC)/high-fat (HF) diet containing cholate, lesion area was 40% lower than in non-transgenic controls; no significant differences in plasma cholesterol or lipoproteins between the experimental groups were observed under these conditions.

Studies by Isoda *et al.*^{17,18} further clarified the role of IL-1Ra in atherosclerotic lesion development. The studies focused on the comparison of atherosclerotic lesion between IL-1Ra^{+/+}/ApoE^{-/-} and IL-1Ra^{+/-}/ApoE^{-/-}-mice, because IL-1Ra^{-/-}/ApoE^{-/-}-mice were significantly leaner, had significantly elevated total plasma cholesterol, decreased HDL-cholesterol levels, and severe fatty livers when compared with the other mice. The lowering of serum levels of IL-1Ra in IL-1Ra^{+/-}/ApoE^{-/-}-mice to approximately half of those in IL-1Ra^{+/+}/ApoE^{-/-} was associated with a significant increase in lesion development during early atherogenesis. At later stages, the differences of lesion size between these mice disappeared, but, most importantly, (lack of) IL-1Ra appeared to have modulated plaque composition during lesion progression, with a significant increase in macrophages and depletion of smooth muscle cells (SMC) in IL-1Ra^{+/-}/ApoE^{-/-}, suggesting reduced plaque stability.

Merhi-Soussi *et al.*¹⁵ also noticed massive vascular inflammation in IL-1Ra^{-/-}/ApoE^{-/-} mice with marked macrophage infiltration in the adventitia and severe destruction of elastic lamina, paralleled by severe illness and high death rate.

Taken together, the reported effects of IL-1Ra on early lesion development and plaque composition point to an important role of IL-1 signalling in atherogenesis. In line, IL-1 β ^{-/-}/ApoE^{-/-} showed a significant 30% decrease in atherosclerosis at 12 and 24 weeks of age compared with that in IL-1 β -expressing ApoE^{-/-} mice.¹⁹ There was no significant difference in plasma lipid levels between the two genotypes. Kamari *et al.*²⁰ confirmed these observations using IL-1 β ^{-/-}/C57/BL6-mice and additionally demonstrated that genetic deletion of IL-1 α reduced lesion area by 56%. Of note, IL-1 α ^{-/-} mice displayed high levels of non-HDL-cholesterol whereas IL-1 β ^{-/-}-mice had higher

levels of HDL-cholesterol. Transplantation of IL-1 α ^{-/-} or IL-1 β ^{-/-} bone marrow (BM) in irradiated C57/BL6-mice reduced early lesion formation by 59 and 33%, respectively, when compared with wild-type BM transplanted mice, without changing plasma lipids.²⁰

The evidence for an important role of IL-1 signalling in atherosclerosis is further strengthened by a study by Chi *et al.*²¹ who showed that the absence of IL-1R markedly reduces the progression of atherosclerosis in ApoE^{+/-}-mice under experimental conditions known to favour the aggravation of vascular lesions, i.e. 24 weeks of exposure to HF-diet and *P. gingivalis*. The effect of IL-1R absence persisted whether the inciting factors were genetic, dietary, or infectious, alone or in combination, thus highlighting the prominent role of IL-1 signalling in the atherosclerotic process and emphasizing its attractiveness as a therapeutic target.

3. Interleukin-2

IL-2 is a pro-inflammatory cytokine produced by T_H1 lymphocytes. Intraperitoneal injection of IL-2 into ApoE^{-/-}-mice fed with HF-diet enhances atherosclerosis, while anti-IL-2 antibody treatment has a protective effect, indicating that IL-2 is atherogenic.²²

4. Interleukin-3

See below under CSFs.

5. Interleukin-4

IL-4, a T_H2 cytokine with a key role in T_H2-cell differentiation, is produced by activated T-lymphocytes and mast cells, and can exert both pro- and anti-inflammatory effects.^{9,23,24}

The role of IL-4 in atherosclerosis has been investigated in several mouse models (Table 1).^{23,25-27}

Davenport and Tipping²³ found that IL-4^{-/-}/ApoE^{-/-}-mice had a significant reduction in plaque area in the aortic root compared to ApoE^{-/-}-mice at 30 weeks of age. At 45 weeks, there were no significant differences in lesion sizes in the aortic root between the strains; however, IL-4^{-/-}/ApoE^{-/-}-mice showed a marked decrease in atherosclerosis in their aortic arch compared to ApoE^{-/-} mice.

King²⁵ demonstrated that immune cell-specific deficiency of IL-4 in hypercholesterolemic, cholate-fed female LDLR^{-/-}-mice, produced by repopulation with BM stem cells from IL-4^{-/-} mice, led to a reduction in atherosclerosis in the arch/thoracic regions of the aorta, but not in the aortic root. The same group reported no effect of genetic IL-4-depletion in models of cholesterol-induced atherosclerosis (female LDLR^{-/-} mice; after 4 weeks), angiotensin-II-induced atherosclerosis (male ApoE^{-/-} mice; after 4 weeks), and spontaneous atherogenesis (male and female ApoE^{-/-} mice; up to 36 weeks).²⁷ I.p. administration of mouse IL-4 protein during 30 days had also no effect on atherogenesis in ApoE^{-/-} fed a normal chow or a HF-diet.²⁷ In none of these experiments modulation of IL-4 altered plasma cholesterol.

Using a model of accelerated fatty streak formation induced by heat-shock-protein-65 or *Mycobacterium tuberculosis*, George *et al.*²⁶ found that fatty streak formation in IL-4^{-/-} mice was significantly reduced compared with lesions in wild-type C57/BL6 mice, lending support to a pro-atherogenic character of IL-4.

However, in another study by the same group, fatty streak formation in the aortic root of C57/BL6 fed a HC-diet was not influenced by IL-4 deficiency.²⁴ Notably, the lesions in this study were not as advanced as in the above studies using ApoE^{-/-}.

At odd with these findings, IL-4 injection decreased aortic lesion size in mice under conditions of mild hypercholesterolemia, suggesting that exogenous and endogenous IL-4 may have opposite effects.²⁸

Overall, the data show that IL-4 can play a role in the development of atherosclerosis at various vascular sites. The net effect of IL-4 may depend on the disease stage, reflecting its dual, i.e. pro- and anti-inflammatory, character.

6. Interleukin-5

IL-5 is mainly produced by T_H2 cells and mast cells. In LDLR^{-/-} mice which were reconstituted with wild-type or IL-5^{-/-} BM cells, Binder *et al.*²⁹ established that IL-5 links adaptive and natural immunity specific for epitopes of oxidized low-density lipoprotein (oxLDL) and had an overall atheroprotective role.

7. Interleukin-6

IL-6 is a multifunctional cytokine that regulates various aspects of the immune response, acute-phase reaction, and haematopoiesis. IL-6 is inducible by IL-1, and consequently concentrations in serum are often a reflection of IL-1 activity *in vivo*. Nonetheless, IL-6 has been identified as an independent risk factor for coronary artery disease. Direct involvement of IL-6 in atherosclerotic lesion development was suggested by experiments in which mice had been treated for 6–21 weeks with recombinant IL-6 (rIL-6).³⁰ rIL-6 treatment increased lesion size in HF/cholate-fed C57/BL6 and ApoE^{-/-} mice 5.1- and 1.9-fold, respectively when compared to lesions in saline-treated animals (Table 1). Total cholesterol levels were unchanged between rIL-6-treated and non-treated groups. Seemingly contrasting these observations with supra-physiological injections of rIL-6 is a study reporting that IL-6^{-/-} mice developed 1.7 times larger fatty streak lesions than C57/BL6 after 15 weeks on atherogenic Paigen diet.³¹

In another study, using chow-fed ApoE^{-/-} mice that were IL-6 mono- or double-deficient, there was a tendency for decreased fatty streak formation when compared with ApoE^{-/-} mice, despite a significant increase in plasma non-HDL cholesterol in IL-6^{-/-}/ApoE^{-/-}.³² However, when female IL-6^{-/-}/ApoE^{-/-} mice were maintained for 1 year on a normal mouse-chow, the animals showed similar hypercholesterolemia to IL-6^{+/+}/ApoE^{-/-} but presented significantly larger and more calcified lesions.³²

In a very similar study, Schieffer *et al.*³³ used male IL-6^{-/-}/ApoE^{-/-} double knockout mice on a normal chow diet for 1 year. A lifetime deficiency of IL-6 in the male ApoE^{-/-} model resulted in enhanced atherosclerotic lesion formation, similarly as reported by Elhage *et al.*,³² but significantly higher levels of total cholesterol, LDL, and VLDL, which is deviant from the findings with female IL-6^{-/-}/ApoE^{-/-}. Schieffer *et al.* also reported reduced collagen metabolism and decreased recruitment of inflammatory cells to the atherosclerotic plaque in male IL-6^{-/-}/ApoE^{-/-} mice.

Table 1 Summary of the reviewed atherosclerosis studies and their experimental conditions, i.e. mouse model, gender, composition of diet, animal age, and treatment period, effect on cholesterol, atherosclerotic lesion size, and lesion composition

Cytokine	Mouse model (treatment)	Diet		Age at start of treatment + treatment period	Cholesterol	Lesion size	Composition	Reference
IL-1Ra	ApoE ^{-/-} (M/F)+rec hu IL-1Ra	HFD+cholate	16% fat/1.16% chol/ 0.5% cholate	2 m + 1 m	↔	56%↓(M)	Fatty streak	14
	s human IL-1Ra Tg/ApoE ^{-/-} (M)	HFD	1.25% chol	10 w + 10 w	↔	63%↓(F) 47% ↓/53% ↓		15
	ic human IL-1Ra Tg/ApoE ^{-/-} (M)	HFD	1.25% chol	10 w + 10 w	↔	40% ↓/ 67% ↓		15
	IL-1Ra ^{-/-} /ApoE ^{-/-} (M)	HFD	1.25% chol	10 w + 7 w	42% ↓	No atherosclerosis; massive inflammation		15
	IL-1Ra ^{-/-} /C57Bl/6J	HFD	7.5% fat/1.25% chol/ 0.5% cholate	4 w + 12 w	3 x ↓	Tendency to increased foam cell lesion area		16
	IL-1RaTg/LDLR ^{-/-}	HC/HF HC/ HF+cholate		4 w + 10 w 4 w + 4 w	40% ↑ ↔	↔ 40% ↓		16
	IL-1R ^{-/-} /ApoE ^{+/+} vs. IL-1R ^{+/+} /ApoE ^{+/+}	HFD+cholate	15% fat/1.25% chol/ 0.5% cholate	10 w + 14/24 w		14 w: ↔	Milder	21
	IL-1R ^{-/-} /ApoE ^{+/+} vs. IL-1R ^{+/+} /ApoE ^{+/+}	Chow	0.02% chol/ 4.5% fat	10 w + 14/24 w		24 w: 14x↓ 14 w: ↔	Milder	21
IL-1α	IL-1R ^{+/+} /ApoE ^{-/-} vs. IL-1R ^{+/+} /ApoE ^{-/-} (vs. IL-1R ^{-/-} /ApoE ^{-/-})	Chow	0.02% chol/ 4.5% fat	16 w + 24 w	↔	24 w: ~3x↓ n.s. 16 w: 30%↑	A-SMA↓	17,18
	IL-1α ^{-/-} C57BL/6	HF/HC	17% fat/1.25% chol/ 0.5% cholate	24 w 6 w + 19 w	↑ ↑ (non-HDL)	↑ 56% ↓	MOMA-2↑	20
	C57BL/6+IL-1α ^{-/-} BMT	HF/HC	17% fat/1.25% chol/ 0.5% cholate	6 w + 16 w	↔	59% ↓		20
IL-1β	IL-1β ^{-/-} /ApoE ^{-/-} (M)	Chow	4.6% fat/ <0.02% chol	0 w + 12/24 w	↔	33%↓/32%↓		19
	IL-1β ^{-/-} C57BL/6	HF/HC	17% fat/1.25%chol/ 0.5% cholate	6 w + 19 w	↑ (HDL)	50% ↓		20
	C57BL/6+IL-1β ^{-/-} BMT	HF/HC	17%fat/1.25% chol/ 0.5% cholate	6 w + 16 w	↔	33% ↓		20
IL-2	ApoE ^{-/-} (M)+rmlL-2 ApoE ^{-/-} (M)+α-mouse IL-2	HF	21% fat/ 0.15% chol	6 w	ND	↑ ↓	More advanced Less advanced	22
IL-4	IL-4 ^{-/-} /ApoE ^{-/-} (M+F)	Chow		6, 15, 30, 45 w	↔	30 w:27%↓ 45 w: ↓		23
	IL-4T KO/C57BL/6J	Paigen	17% fat/ 1.25 chol	15 w	↔	↔		24

	IL-4 ^{-/-} /C57BL/6J+HSP65 (F)	HF Teklad diet (TD 90221)		15 w	↔	↓		26
	LDLR ^{-/-} +IL-4 ^{-/-} BMT	HF	21% fat/1.25% chol/0.5% cholate	4 w	↔	Root: ↔	↔	25
	LDLR ^{-/-} /IL-4 ^{-/-} (F)	HF Teklad 88137 or Teklad 90221	21% butter fat/0.15% chol	8-10 w + 4 w	↔	Arch: 69%↓	↔	27
	ApoE ^{-/-} /IL-4 ^{-/-} (M, F)	Chow or HF/HC	21% butter fat/0.15% chol	8-10 w + 36 w	↔	↔		27
	ApoE ^{-/-} +i.p. IL-4	Normal chow or HF Teklad 88137	21% butter fat/0.15% chol	8 w + 30 d	↔	↔		27
	C57BL/6J+IL-4 (M)	Teklad diet 96354	20% fat/ 1.5% chol/0.5% cholate	15 w	↔	90%↓		28
IL-5	LDLR ^{-/-} +IL-5 ^{-/-} BMT	Teklad diet 94059	15.8% fat/1.25% chol	16 w	ND	Root: ↑		29
IL-6	C57Bl/6 (M)+rec mouse IL-6	HFD+cholate	20% fat/1.5% chol/0.5% cholate	3 w + 21 w	↔	Arch: ↑	5.1 x ↑	30
	ApoE ^{-/-} (M)+rec mouse IL-6	LFD (chow)	5.67% fat/0% chol	3 w + 21 w	↔	2.4 x ↑		30
	ApoE ^{-/-} (M)+rec mouse IL-6	HFD+cholate	20% fat/1.5% chol/0.5% cholate	3 w + 6 w	↔	1.9 x ↑		30
	IL-6 ^{+/-} /ApoE ^{-/-} (F)	LFD (chow)		0 w + 16 w	↔	28%↓(n.s.)		32
	IL-6 ^{-/-} /ApoE ^{-/-} (F)	LFD (chow)		0 w + 16 w	26%↑(sign.)	29%↓(n.s.)		32
	IL-6 ^{-/-} /ApoE ^{-/-} (F)	LFD (chow)		0 w + 1 y	↔	1.9 x ↑	Calcification area: 4.1 x ↑	32
	IL-6 ^{-/-} /C57Bl/6 (F)	Paigen	15% fat/1.25% chol/0.5% cholate	8-10 w + 15 w	ND	70%↑		31
	IL-6 ^{-/-} /LDLR ^{-/-} (M, F)	Chow		5 w + 16 w	↔	↔	M-45% (n.s.) F-38% (n.s.)	34
	IL-6 ^{-/-} /LDLR ^{-/-} (M, F)	Western-type diet	20% fat/1.5% chol	5 w + 16 w	↔	↔	M-27% (n.s.)	34
	IL-6 ^{-/-} /LDLR ^{-/-} (M, F)	Paigen diet	15% fat/1.25% chol/0.5% cholate	5 w + 16 w	↔	↔	F-19% (n.s.) M-15% (n.s.)	34
	IL-6 ^{-/-} /ApoE ^{-/-} (M)	Chow		0 w + 53 ± 4 w	60% ↑	1.4-1.9 x ↑	F-22% (n.s.)	33
IL-10	mIL-10TG/C57BL/6J	HF	15% fat/1.25% chol/0.5%cholate	15 w	↔	2.5x↓		40
	IL-10 ^{-/-} /C57BL6	HF	15% fat/1.25% chol/0.5%cholate	15 w	HDL↓	2x↑	Fatty streak	40
	LDLR ^{-/-} +IL-10Tg BMT	HF (TD94059)	15.8% fat/1.25% chol	20 w	↔	2x↓		44
	IL-10 ^{-/-} /ApoE ^{-/-} (M+F)	Chow		16 w + 48 w	total chol ↔	16 w:3x↑	SMC+inflammatory cells	95
					VLDL↓LDL↑	48 w: ↔	↔	

Continued

Table 1 Continued

Cytokine	Mouse model (treatment)	Diet		Age at start of treatment + treatment period	Cholesterol	Lesion size	Composition	Reference
	IL-10 ^{-/-} / C57BL/6J (F)	HF	18% fat/1.25% chol/ 0.5% cholate	8 w + 16 w	↔	SPF:3x↑ CONV:30x↑	Macrophages ↔ T-cells 2.5x↑ Collagen ↓	41
	LDLR ^{-/-} +IL-10 ^{-/-} BMT	HF	15% fat/1.25% chol	14 w	↔	35%↑	SMC ↔ Collagen 49%↓ Macrophage 62%↑ T-cells 116%↑	45
	ApoE ^{-/-} +mIL-10-HVJ (M)	HF/HC	7.5% fat/1.25% chol/0.5% cholate	9 w	↔	2.5x↓	Macrophages 2x↓ T-cells 2x↑ Collagen ↔	42
	ApoE ^{-/-} +AAV2/5-mIL-10	HF		8 w	chol+TG ↓	31%↓	MCP-1 ↓	43
	LDLR ^{-/-} +AAV2-hIL-10	HC	2%chol	18 w		↓	Macrophages ↓ Nitrotyrosine ↓	39
IL-12	IL-12 ^{-/-} /ApoE ^{-/-} (M+F)	Chow		6, 15, 30, 45 w	↔	30 w:52%↓ 45 w:↔	30 w: fewer macrophages	23
	ApoE ^{-/-} +recmIL-12 (M)	Chow		30 d	↔	↑	More advanced CD3+ cells ↑	47
	LDLR ^{-/-} +IL-12-PADRE (F)	Western-type diet	15% fat/0.25% chol	2 w diet +6 w collar	↔	68.5%↓	4.2x↑ SM-actin 2.8x↑ collagen Macrophages ↔	48
IL-18	ApoE ^{-/-} +IL-18	Chow		30 d	↔	2.2x↑	T-cells 4.5x↑ MHCII+cells 4.4x↑	49
	IFN-γ ^{-/-} /ApoE ^{-/-} +IL-18	Chow		30 d	↔	↔	↔	77
	IL-18 ^{-/-} /ApoE ^{-/-}			24 w	50%↑	35%↓	I-A ^b gene ↓ CD4+ cells↓ SMactin ↑	50
	ApoE ^{-/-} +pcDNA3-mIL-18BP (M)			9 w	↔	24%↓	Macrophages 50%↓ T-cells 67%↓ 2x↑SMC 85%↑ collagen TUNEL 3.5x↓	51
IL-20	ApoE ^{-/-} +intramuscular electroporation of IL-20 (F)	Atherogenic diet	0.15% chol	10 w	ND	1.4x↑	Macrophages ↔ Mig, I-TAC, TNF-α, IL-6 ↑	54
TNFR	p55 ^{-/-} /ApoE ^{-/-}	Chow		0 w + 64 w	↔	↔	↔	57
	p55 ^{-/-} or wt grafts in ApoE ^{-/-}	Chow	9% fat	6 w + 8 w post operatively	not reported	Lumen 31%↑	SMC proliferation ↓	58
	p55 ^{-/-} /ApoE ^{-/-} (F)	Chow	9% fat	60 w	↔	12%↓		58
	p55 ^{-/-} /C57Bl6(c.pneumoniae infected)	HF/HC	15% fat/ 1.25% chol/ 0.5% cholate	9 w + 12/16 w	↔	↔		59

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	p55 ^{-/-} /C57Bl6 (F)	HF/HC	15% fat/ 1.25% chol/ 0.5% cholate	6-8 w + 14 w	↔	2.3x↑	↔	55
	p55 ^{-/-} / p75 ^{-/-} / p55 ^{-/-} p75 ^{-/-} /C57Bl6	HF/HC	15% fat/ 1.25% chol/ 0.5% cholate	6 w + 18 w	↔	2.4x↑ / ↔ / 2.4x↑		56
TNF-α	TNF ^{-/-} /E3Leiden (F)	Western-type diet	15% fat/ 1% chol	8 w + 20 w	↔	↔	Early↑ necrosis↓ Necrosis ↑ Apoptosis ↑	64,65
	TNFα ^{-/-} /ApoE ^{-/-}	Western-type diet	21% fat/ 0.15% chol	4 w + 10/40 w	↑n.s./ ↔	50%↓ / 60%↓		60
	TNFα ^{-/-} /ApoE ^{-/-} (BMT)	Western-type diet	21% fat/ 0.15% chol	10 w + 25 w	↔	83%↓		60
	TNFα ^{-/-} /ApoE ^{-/-} (murine sTNF-R1/Fc chimera)	Chow		7 w + 25 w	↑n.s.	75%↓		60
	p55 ^{-/-} /ApoE ^{-/-}	Chow		0 w + 64 w	↔	↔	↔	57
	p55 ^{-/-} (M)	Atherogenic diet	15% fat/ 1.25% chol/ 0.5%cholate	9 w + 12/16 w	↔	↔		59
	p55 ^{-/-} /ApoE ^{-/-} (F)	Atherogenic diet	15% fat/ 1.25% chol/ 0.5% cholate	6-8 w + 14 w	↔	Lesion size 2.3x↑	Cell number↓ Scavenger receptor expression↑	55
	TNFα ^{-/-} /C57BL/6 (F)	HF/HC	15% fat/ 1.25% chol/ 0.5% cholate	6 w + 16 w	46%↑	↔		56
	TNFα ^{-/-} /ApoE ^{-/-} (M+F)	Chow		0 w + 12 w	↔	27%↓		61
	TNF ^{-/-} /C57BL/6 (M)	HF/HC	17% fat/ 1.25% chol/ 0.5% cholate	8-10 w + 20 w	61.5%↑HDL	17.8x↓		65
	TNF ^{-/-} / ApoE ^{-/-} (F)	HF/HC	17% cocoa butter, 1.25% cholesterol	6 w + 26 w	16.9%↓IDL/LDL 21.6%↓VLDL not reported	30%↓	Reduced wall-thickness	62
	tmTNF/C57Bl6	HF/HC	17% fat/ 1.25% chol/ 0.5% cholate	8-10 w + 20 w	↔	1.8x↓n.s.		65
Anti-TNF	ApoE ^{-/-} +TNFbp (M+F)	HF/HC	16% fat/1.15% chol/ 0.5% cholate	8 w + 4 w	↔	M ↔		14
IFN-γ	IFNγ ^{-/-} /ApoE ^{-/-} (M+F)	Chow		36 w	↔	F: 35%↓ n.s. M: 42%↓	Neutral lipid, macrophages, T-cells, collagen ↔	75
		HF (TD88137)	0.15% chol/20% butterfat	8 w + 12 w	↔	F: ↔ M: 74%↓	Neutral lipid, macrophages, T-cells, collagen ↔	
	ApoE ^{-/-} (M)+mIFNγ IP	Chow		16 w + 4 w	15%↓VLDL	F: ↔ 2x↑	2,7x↑ T-cells 3.3x↑ MHCII ⁺ cells	76
	IFN-γR ^{-/-} /ApoE ^{-/-} (F)	Western-type diet	21% fat/0.15% chol	5 w + 12 w	FC↑	59%↓ lipid content	Smaller	53

Continued

Table 1 Continued

Cytokine	Mouse model (treatment)	Diet		Age at start of treatment + treatment period	Cholesterol	Lesion size	Composition	Reference
							phospholipid-rich particles↑ apoA-IV↑	
	IFN- γ ^{-/-} /LDLr ^{-/-} (M+F)	D12108 research diets	40% lipid/ 1.25% chol	5-6 w + 8 w	↔	75%↓ lipid area	Less cellular Collagen↑ T-cells↔	74
				5-6 w + 20 w	↔	62%↓ lesion area En face 46%↓ 43%↓ lipid area Lesion area ↔ en face 70%↓	Macrophages↓ MHCII ⁺ cells ↓ Collagen↓ ↔ MHCII ⁺ cells ↓	
Anti-IFN- γ	ApoE ^{-/-} + siFN- γ R plasmid	Western-type diet	20% fat/ 0.15% chol	8 w + 4 w	↔	en face plaque area 60%↓	IFN- γ , IL-1 β , MCP-1, VCAM-1↓	78
		Western-type diet	20% fat/ 0.15% chol	8 w + 8 w	↔	Attenuate lesion progression 50%, stabilization	Inflammatory cyto/chemokines↓	96
				8 w + 12 w	↔	Attenuate lesion progression 40%	MMP-9/13↓ Collagen I↑ pSTAT1/STAT1↓	
MIF	ApoE ^{-/-} + α -MIF mAb	Chow		? + 14 w	↔	Lesion area %↓ n.s.	CD40/CD40L↓ 4x↓ Macrophages	71
	ApoE ^{-/-} + α -MIF Ab	Atherogenic diet		? + 16 w		Aortic root surface↓	Inflammatory mediators↓ T-cells↓	2
	Mif ^{-/-} /LDLr ^{-/-}	Chow		0 w + 30 w			Macrophages↓ Monocyte adhesion↓ Macrophages↓	2
	Mif ^{-/-} /LDLr ^{-/-} (M)	Atherogenic diet		6 w + 12/26 w	LDL↓	26 w: En face lipid deposition 4.5x↓	Ki67+ cells↓	70
					HDL↔	Longitudinal intima 7x↓	SMCs↓ n.s.	
	ApoE ^{-/-} + MIF mAb + carotid transluminal wire injury	Atherogenic diet	21% fat	8 w + 3 w	TG↓ ↔	Neointima/media volumes↔	Collagen↓ n.s. Macrophages 2x↓	72

	LDLr ^{-/-} + α -MIF mAb+carotid injury	HF	20.1% fat/1.25% chol	Weaning+12 w	\leftrightarrow	Neointimal thickening 54% \downarrow	SMC2x \uparrow Proliferation 56% \downarrow	73
M-CSF	Op0/op1/op2/ ApoE ^{-/-} (M+F)	Chow		0 w + 16 w	Op1 \uparrow	Op1 \downarrow	CD45+ cells 27% \downarrow Apoptosis \uparrow	85
	Op0/op1/op2/ ApoE ^{-/-} (M+F)	Chow		12 w + 12 w	Op0 $\uparrow\uparrow$ VLDL Op0: 2.5x \uparrow HDL \leftrightarrow	Op0 $\downarrow\downarrow$		86
	Op0/op1/op2/C57Bl6	Atherogenic diet	15% fat/ 1.25% chol/ 0.5% cholic acid	24 w + 15 w	Op0: 2x \uparrow	Op0: 84% \downarrow		86
	Op0/op1/op2/ ApoE ^{-/-} (M+F)	Western-type diet	42% fat/ 0.15% chol	3 w + 9 w	Op1:3x \uparrow Op0: 1.5x \uparrow	Op1: 46% \downarrow Op0: 4x \downarrow	Monocytes 3x \downarrow	4
	Op/+, op/op/ LDLr ^{-/-} (M+F)	Chow Atherogenic diet	4.5% fat/0.02% chol 15% fat/ 1.25% chol/ 0.5% cholic acid	4 w + 12 w 12 w + 16 w	Op/op 25% \uparrow	Op0: \downarrow Op/op 99.7% \downarrow	Macrophages \downarrow	5
	ApoE ^{-/-} (F)+murine c-fms mAb	HF	20% fat/ 0.3% chol	6 w + 6 w diet+6 w Ab	Op/+16% \uparrow \leftrightarrow	Op/+99.4% \downarrow 70% \downarrow		87
				6 w + 12 w diet + 6 w Ab	\leftrightarrow	\leftrightarrow	Macrophages \leftrightarrow SMC \leftrightarrow	
GM-CSF	GM-CSF ^{-/-} , GM-CSF ^{+/-} / LDLr ^{-/-} (M+F)	Western-type diet	TD88137, teklad	10 w + 12/13 w	\leftrightarrow	- / - : ~20% \downarrow + / - : \downarrow	Macrophages \leftrightarrow	88
	ApoE ^{-/-} (M)+GM-CSF (10 μ g/ kg ⁻¹ d ⁻¹ ; 5 d per w)	Paigen diet	30 kcal % fat/ 1.25% chol/0.5% cholate	8 w + 8 w	\leftrightarrow	2x \uparrow	SMC \leftrightarrow Collagen \leftrightarrow Elastin \downarrow CD11c+ cells 63% \downarrow CD4+ T _H cells 50% \downarrow IL-6/MCP-1 \leftrightarrow	89
G-CSF	GM-CSF ^{-/-} /ApoE ^{-/-} (M)	HF	20% fat/0.125% chol	8 w + 12 w	\leftrightarrow	30% \uparrow	Macrophages 2x \uparrow SMC \leftrightarrow Collagen 15% \downarrow PPAR γ 50% \downarrow ABCA1, SR-A, CD36 \downarrow	90
	ApoE ^{-/-} (M)+G-CSF (10 μ g/ kg ⁻¹ d ⁻¹ ; 5 d per w)	Paigen diet	30 kcal % fat/ 1.25% chol/0.5% cholate	8 w + 8 w	\leftrightarrow	44% \uparrow	\leftrightarrow	89

To re-examine the hypothesis that IL-6 and the acute-phase-response play an important role in atherogenesis, Song and Schindler³⁴ crossbred IL-6^{-/-} mice with LDLR^{-/-} mice because loss of ApoE protein in ApoE^{-/-} mice has been associated with decreased immune and inflammatory responses.^{11,35} Although there was a trend for modestly smaller lesions in IL-6^{-/-}/LDLR^{-/-} throughout the course of the study of 16 weeks on chow, Western-type, and Paigen diets, these differences never achieved statistical significance. This despite a significant defect in the expression of acute-phase-response genes in IL-6^{-/-}/LDLR^{-/-} Paigen diet fed mice. Similar to the development and structure of atheromata, no significant differences in lipid/lipoprotein were noted, indicating that IL-6 does not contribute significantly either to the regulation of lipid metabolism on chow, Western-type, or Paigen diet fed LDLR^{-/-} mice.

In all, reported studies support both a positive and negative role for IL-6 in the development of atherosclerosis, while some groups failed to identify a substantial role for IL-6 at all in either the development or structure of atheromata. Part of the explanation for the different findings may lie in the experimental set-up (mouse model, male vs. female, diet, age of the animals, duration of the experiment; *Table 1*). Also, IL-6 may have a biphasic effect on the atherosclerotic process as a reflection of its pro-inflammatory or anti-inflammatory nature activities.³⁶⁻³⁸ Furthermore, redundancy may play an important role. IL-6 belongs to a family of cytokines, which include IL-11, leukaemia inhibitory factor, oncostatin M, and ciliary-neurotropic factor, which are expressed in the arterial wall. Since these IL-6-type cytokines all use the same receptor gp130 subunit for signal transduction, they may induce similar (patho)physiological responses as IL-6 and overcome IL-6 deficiency ('redundancy').

8. Interleukin-10

IL-10 is a pleiotropic cytokine produced by a variety of immune cells (e.g. T_H2 cells, macrophages and CD8⁺ cells) that can inhibit a broad array of immune and inflammatory responses.³⁹ Because of its anti-inflammatory properties, IL-10 is thought to be atheroprotective and have anti-atherogenic potential.

Indeed, atherosclerosis is reduced in IL-10-transgenic C57/BL6-mice fed a cholate-containing HF-diet,⁴⁰ whereas their IL-10-deficient counterparts exhibited increased early atherosclerotic lesion formation (*Table 1*).^{40,41} Caligiuri *et al.*⁹⁵ also showed a protective effect of IL-10 by using ApoE^{-/-}/IL-10^{-/-} mice: IL-10-deficiency increased atherosclerosis, LDL, thrombosis, and plaque vulnerability. In line with this, intramuscular gene transfer of IL-10 cDNA reduced atherosclerosis in ApoE^{-/-} mice.⁴² Inhibition of atherogenesis was also found by delivery of adeno-associated virus vector-mediated IL-10 (AAV/IL-10) gene transfer into the tibial muscle of ApoE^{-/-} mice⁴³ or by AAV/IL-10 tail vein injection in LDLR^{-/-} mice.³⁹ Because IL-10 is produced by BM cells, two groups^{44,45} examined the direct role of leukocyte-derived IL-10 on the inflammatory process and development of advanced atherosclerotic lesions in irradiated LDLR^{-/-} mice transplanted with BM cells from IL-10 transgenic,⁴⁴ wild-type⁴⁵ or IL-10^{-/-} mice. After feeding a HF/cholate-free diet, IL-10 deficiency significantly enhanced development of advanced

atherosclerotic lesions, reiterating that leukocytes are a major source of IL-10, active in protection against excessive growth of advanced lesions.

9. Interleukin-12

IL-12 is a heterodimeric (p70) cytokine consisting of a 35 kDa light chain (p35) and a 40 kDa heavy-chain (p40). IL-12 is produced by various inflammatory cell types present in the atherosclerotic vasculature, among which macrophages. Because of its early production in response to stimuli and its ability to enhance T_H1-cell differentiation, IL-12 forms a bridge between innate and adaptive immunity.⁴⁶

IL-12 p40^{-/-}/ApoE^{-/-} mice show markedly less atherosclerosis in the aortic root at 30 weeks of age than ApoE^{-/-} controls (*Table 1*).²³ In addition, daily administration of IL-12 protein promotes atherosclerosis in young ApoE^{-/-} mice compared with control-treated mice.⁴⁷ Hauer *et al.*⁴⁸ reported that functional blockade of endogenous IL-12 by vaccination resulted in a significant 69% reduction of atherogenesis induced in carotid arteries by bilateral perivascular collar placement using LDLR^{-/-} mice; vaccination against IL-12 also improved parameters for plaque stability.

These studies, in which the pro-atherosclerotic role of IL-12 is described, indicate that IL-12 may be a suitable target in the treatment of atherosclerosis.

10. Interleukin-18

IL-18 and IL-1 β are members of the same structural family (IL-1 family). Animal experiments support the concept that IL-18 participates in the pathogenesis of atherosclerosis (*Table 1*). When injected for 30 days with IL-18, ApoE^{-/-} mice exhibit a doubling of the lesion size through release of the pro-inflammatory cytokine IFN- γ , and without a change in serum cholesterol.⁴⁹

Elhage *et al.*⁵⁰ found reduced atherosclerosis in IL-18-deficient ApoE^{-/-} mice, despite an increase in serum cholesterol. Overexpression of the IL-18-binding protein, a naturally occurring, specific inhibitor of IL-18, prevents the spontaneous development of atherosclerosis in ApoE^{-/-} mice,⁵¹ thus confirming the pro-atherogenic character of IL-18. Part of the pro-atherogenic effect of IL-18 may be indirect since IL-18, in particular in combination with IL-12, is a potent inducer of the production of IFN- γ , which has aggravating effects on atherosclerosis (see below).^{52,53}

11. Interleukin-20

IL-20 belongs to the IL-10 family and is a proinflammatory cytokine, preferentially expressed in monocytes. Systemic delivery of IL-20 by intramuscular electroporation of IL-20 expression vector resulted in increased atherosclerotic lesion areas in ApoE^{-/-} mice, suggesting that IL-20 acts as a pro-atherogenic cytokine.⁵⁴

12. Tumour necrosis factor- α

TNF- α is a pleiotropic cytokine that exerts potent pro-inflammatory effects in atherosclerosis and other metabolic and inflammatory disorders such as obesity and insulin resistance which are also risk factors for cardiovascular diseases. TNF- α is primarily produced by monocytes and

macrophages. The involvement of TNF- α in the pathogenesis of atherosclerosis is supported by its presence in human atherosclerotic plaques. Furthermore, circulating TNF- α levels are associated with increased risk of recurrent myocardial infarction, atherosclerotic thickening of carotid intima-media, disturbances in triglyceride and glucose homeostasis, and with age-related atherosclerosis.

Lymphotoxin- α (LT α) is another member of the TNF ligand family and is synthesized predominantly by activated T- and B-lymphocytes. Like TNF- α , LT α is expressed in atherosclerotic plaques. TNF- α and LT α elicit responses through two receptors termed p55 (TNFR1) and p75 (TNFR2), of which p55 activates the majority of biological responses. The role of TNF- α (and LT α) in atherosclerosis is not fully clear at present because results in animal models are controversial (Table 1).

In female C57/BL6 wild type mice lacking p55 (p55^{-/-} mice), Schreyer *et al.* found an unexpected 2.3-fold increase in atherosclerosis when feeding the mice an atherogenic diet.⁵⁵ In a subsequent study, the same group found that loss of p75 did not alter lesion development, and loss of both p55 and p75 resulted in lesion areas comparable with those for p55^{-/-} mice.⁵⁶ No significant changes in plasma lipid levels were observed between genotypes. By contrast, in older p55^{-/-} mice on an ApoE^{-/-} background no effect on lesion progression or composition of very advanced plaques was observed.⁵⁷ Using an arterial grafting model (i.e. transplantation of lesion-free wild-type- or p55^{-/-}-donor carotid grafts in ApoE^{-/-} recipient mice) Zhang *et al.* demonstrated that expression of p55 in just the arterial wall (SMC and EC) contributed substantially to both early-stage and late-stage atherosclerosis by enhancing adhesion molecule and chemokine expression as well as SMC proliferation.⁵⁸ The authors also analyzed atherosclerosis in 60-week-old p55^{-/-}/ApoE^{-/-} and ApoE^{-/-} mice and showed that p55-deficiency results in 12% less thoracic atherosclerosis at comparable cholesterol levels.

Campbell *et al.* also reported diminished atherosclerotic lesion development in male p55^{-/-} mice fed a HF/HC-diet.⁵⁹ In addition, these investigators showed that signalling through the p55 receptor may also play a role in the atherogenic effects of *C. pneumoniae* in hyperlipidaemic mice. Whereas *C. pneumoniae*-infected male C57/BL6J-mice on HF/HC-diet showed a 2.5- to 3.3-fold enlarged lesion size in the aortic sinus in comparison to uninfected mice, no acceleration of atherosclerotic lesion development was observed in infected p55^{-/-} mice compared to uninfected controls. No differences in total plasma cholesterol values were observed between infected and sham-inoculated animals.

Elhage *et al.*¹⁴ used repeated subcutaneous injections of a specific TNF/LT inhibitor (TNF-binding-protein; TNFbp) consisting of two molecules of the extracellular domain of p55 linked to a polyethylene glycol molecule, which binds with equal affinity to both TNF- α and LT α . This treatment of ApoE^{-/-} mice with TNFbp did not affect total plasma cholesterol, but there was a tendency to a decrease in fatty streak area in females, not in males. In this study, however, the duration of TNFbp treatment was relatively short (1 month) and the cytokine antagonist was delivered subcutaneously.

Involvement of TNF- α and/or LT α in the progression of atherosclerosis was further substantiated by Branen

*et al.*⁶⁰ Mice deficient in both ApoE and TNF- α exhibited a 50% reduction in lesion size after 10 weeks of Western-style diet feeding. In ApoE^{-/-} mice treated with recombinant soluble p55-receptor-releasing pellets, the lesion size was 75% reduced (after 25 weeks).

Similarly, Ohta *et al.*⁶¹ reported that disruption of the TNF- α gene diminishes atherosclerosis development in ApoE^{-/-} mice without affecting serum cholesterol. Kober *et al.*⁶² confirmed this observation and further demonstrated by MRI that lesion progression and atherosclerotic wall-thickening is reduced in TNF^{-/-}/ApoE^{-/-} mice.

Chew *et al.*⁶³ administered thalidomide, an inhibitor of TNF- α production, to female ApoE^{-/-} mice fed a HF Western-type diet without cholate and reported a significantly smaller lesion size by thalidomide as compared to placebo-treatment.

Boesten *et al.*,⁶⁴ using TNF- α -deficient ApoE*3Leiden mice fed a cholesterol-rich diet, found that the absence of TNF- α did not affect atherosclerotic lesion area, but inhibited lesion progression towards an advanced phenotype.

Canault *et al.*⁶⁵ investigated the effect of a transmembrane form of TNF- α on atherosclerosis in male mice. They compared the development of early atherosclerotic lesions in (i) TNF^{-/-} mice that express only a non-cleavable transmembrane form of TNF (tmTNF mice), (ii) wild-type C57/BL6 (WT) mice, and (iii) TNF^{-/-} mice, all fed an atherogenic diet for 20 weeks. Lipid deposition was most prominent in WT, tended to be lower in tmTNF mice, and rare in TNF^{-/-} mice. Macrophage accumulation was five-fold lower in tmTNF than in WT mice.

In WT mice, the plasma lipid profile was more atherogenic than that of TNF^{-/-} mice, but not significantly different from that of tmTNF mice. These results indicate that in contrast to TNF^{-/-} mice, mice expressing exclusively tmTNF are not completely protected from early atherosclerotic lesion formation, although their lesions have a less inflammatory state than those of WT mice, which underlines the stronger pro-inflammatory potential of soluble TNF.

In contrast to Canault *et al.*, and seemingly at variance with their findings with p55^{-/-} mice,⁵⁵ loss of TNF- α did not alter lesion development in female C57/BL6 mice in experiments described by Schreyer *et al.*, while LT α deficiency resulted in a marked 62% reduction in lesion size.⁵⁶ The disparity in results obtained by Schreyer *et al.* between ligand- and receptor-deficient mice suggests there are undefined members of the TNF ligand and receptor signalling pathway involved with regulating atherogenesis.

13. Macrophage migration inhibitory factor

MIF is a pleiotropic cytokine with hormone-like and enzymatic properties.⁶⁶ Unique among cytokines is MIF's uptake into target cells where it controls cell cycle and inflammation.^{67,68} MIF is predominantly released from macrophages, T-lymphocytes, and SMC, and was shown to be increasingly expressed during atherogenesis,⁶⁹ with oxLDL being a major inducer.

Genetic MIF-deletion in LDLR^{-/-} mice retarded the atherosclerotic process and resulted in reduced intimal thickening, SMC proliferation, lipid deposition, and protease expression (both cathepsins and matrix metalloproteinases)⁷⁰ (Table 1). After 26 weeks on an atherogenic diet, 20% of the MIF^{-/-}/LDLR^{-/-} mice had developed

only early, fatty-streak-like lesions, whereas >80% of the LDLR^{-/-} mice displayed advanced lesions containing calcification and lipid cores. Antibody blockage of MIF significantly reduced (by 75%) intimal macrophage infiltration in ApoE^{-/-} mice undergoing spontaneous atherosclerosis and diminished the aortic plaque area.⁷¹ Schober *et al.*⁷² showed that MIF antibody treatment also diminishes neointimal macrophage/foam cell content after endothelial denudation. In the same study, SMC and collagen content were increased, reflecting a shift in plaque composition towards a stable phenotype. Decreased neointimal thickening and cellular proliferation was also shown in LDLR^{-/-} mice on a C57/BL6J background undergoing carotid injury.⁷³ Seven days after surgery, anti-MIF treatment reduced the number of inflammatory CD45-positive cells invading the intima by 27%. In accordance with this anti-inflammatory effect achieved with MIF antibody treatment, Burger-Kentischer *et al.*⁷¹ reported that immunoneutralization of MIF reduces parameters of aortic (CD40L, ICAM, and MMP-2) and systemic inflammation (SAA, fibrinogen, and IL-6).

A recent study demonstrates that blockage of MIF in ApoE^{-/-} mice with established advanced atherosclerotic lesions leads to plaque regression and reduces monocyte and T-cell content in atherosclerotic lesions.² Using ApoE^{-/-} mice, MIF^{-/-}/LDLR^{-/-} mice, and anti-MIF antibodies, the authors showed that MIF functions as a non-cognate CXCR ligand and plays a prominent role in the recruitment of macrophages, T-cells, and neutrophils. The proatherogenic effects of MIF may thus mainly be explained by its cell arrest and chemoattractant functions.

14. Interferon- γ

The pleiotropic cytokine IFN- γ is a pro-inflammatory mediator that is expressed at high levels in atherosclerotic lesions by various cells, including monocytes/macrophages, T_H1 cells, and natural killer T-cells (NKT).⁶ Activated NKT can produce significant amounts of IFN- γ and are pro-atherogenic in mice (Hansson and Libby¹ and references cited therein). IFN- γ has been implicated in the atherosclerotic process via direct effects^{53,74-76} and indirectly via IL-12 and IL-18^{23,50,77} (Table 1).

Daily injection of recombinant IFN- γ in ApoE^{-/-} mice significantly increased lesion size 2-fold, despite a 15% decrease in serum cholesterol levels.⁷⁶ IFN- γ administration also significantly increased the number of T-cells within lesions. The pro-inflammatory role of IFN- γ was further underscored in studies using IFN- γ ^{-/-}/ApoE^{-/-} mice.⁷⁵ Deficiency of endogenous IFN- γ decreased atherosclerosis development in male ApoE^{-/-} mice fed a normal or HF-diet. This decrease was associated with a decreased abundance and activation of T-cells, without changes in serum cholesterol. Deficiency of IFN- γ has also been shown to substantially decrease extent and influence phenotype of atherosclerosis in male and female LDLR^{-/-} mice, without concomitant changes in lipoprotein profiles.⁷⁴

In line with this, female IFN- γ R^{-/-}/ApoE^{-/-} mice fed a Western-type diet showed a substantial reduction in lesion size, a 60% reduction in lesion lipid accumulation, a decrease in lesion cellularity, and a marked increase in lesion collagen content. Notably, the IFN- γ R^{-/-}/ApoE^{-/-} mice exhibited a significant increase in potentially atheroprotective phospholipid/apoA-IV rich particles.⁵³ Koga

*et al.*⁷⁸ investigated blocking of IFN- γ function by overexpressing a soluble mutant of IFN- γ receptor (sIFN γ R) in ApoE^{-/-} mice and found a reduced luminal plaque area.

There is also indirect evidence for a role of IFN- γ in atherogenesis. One of the primary factors regulating IFN- γ secretion is IL-18. Whitman *et al.*⁷⁷ demonstrated that the absence of IFN- γ in IFN- γ ^{-/-}/ApoE^{-/-} mice ablated the effects of recombinant IL-18 administration on atherosclerosis. Elhage *et al.*⁵⁰ reported that IL-18^{-/-}/ApoE^{-/-} mice exhibited a substantial, 35% reduction in lesion size when compared with IL-18-competent littermates, while the compound knockout mice displayed reduced I-A^b gene expression, implying reduced local IFN- γ stimulation.

IL-18 functions synergistically with other co-stimulators, particularly IL-12, to induce pronounced secretion of IFN- γ by macrophages and T-cells. Compared to ApoE^{-/-} mice, IL-12^{-/-}/ApoE^{-/-} mice displayed a 52% reduction in plaque area in the aortic root at 30 weeks of age,²³ similar to that reported in IFN- γ R^{-/-}/ApoE^{-/-} mice (which also have impaired T_H1 responses).⁵³

15. Colony stimulating factors

CSFs are key cytokines involved in the survival, proliferation, and differentiation of myeloid progenitor cells as well as the functional activation of mature myeloid cells. Four CSFs have been cloned and characterized: multi-CSF/IL-3 (produced by T_H1 and T_H2-cells), granulocyte macrophage-CSF (GM-CSF), macrophage-CSF (M-CSF=CSF-1), and granulocyte-CSF (G-CSF).⁷⁹ The CSFs have a significant overlap in their biological actions. G-CSF and M-CSF are essentially lineage-restricted in their actions on neutrophil and macrophage precursors while GM-CSF and IL-3 also can stimulate other cell types. A comprehensive body of evidence now exists that CSFs have actions in cardiovascular diseases, among which atherosclerosis^{80,81} (Table 1).

M-CSF has been implicated in a variety of inflammatory disorders, including atherosclerosis (see references^{5,80} and references therein). Atherogenic oxidized LDLs induce aortic EC and SMC expression of M-CSF, partly through activation of NF- κ B.^{5,82-84} The localized expression of M-CSF in the vessel wall may be critical in promoting the survival of lipid-laden foam cells observed in early and advanced stages of atherosclerosis. M-CSF selectively regulates the growth and survival of mononuclear phagocytes by binding to a specific cell surface receptor, *c-fms*. In addition, M-CSF functions as a chemotactic factor for monocytes and regulates the effector functions of mature monocytes and macrophages, i.e. it modulates inflammatory responses by stimulating the production of other cytokines (e.g. IL-6, IL-8, IL-12, IL-18, and TNF- α) and growth factors.

Osteopetrotic (*op/op*) mice that lack M-CSF due to a point mutation in the M-CSF gene have proven to be useful in examining the role of M-CSF in atherogenesis. Smith *et al.*⁸⁵ found that *op*/ApoE^{-/-} mice fed a low-fat diet had significantly smaller proximal aortic lesions than their ApoE^{-/-} littermates, despite an almost 3-fold increase in plasma cholesterol. Qiao *et al.*⁸⁶ reported experiments in which atherosclerosis was induced either by feeding *op/op* mice a HF/HC-diet or by crossbreeding these mice with ApoE^{-/-} mice to generate M-CSF^{-/-}/ApoE^{-/-} mice. In both cases, M-CSF deficiency significantly reduced

atherogenesis. The effect of the M-CSF deficiency appears not to be mediated by changes in lipoproteins, because deficient mice exhibited higher levels of atherogenic lipoprotein particles. De Villiers *et al.*⁴ also reported that mice deficient in M-CSF (op) and ApoE display elevated cholesterol levels but are protected from atherosclerosis, thereby illustrating the importance of both M-CSF and M-CSF-dependent monocytes/macrophages in maintaining cholesterol homeostasis and in atherogenesis.

Using LDLR^{-/-} mice, Rajavashisth *et al.*⁵ showed that atheroma development depends on M-CSF concentration, as not only did homozygous op/op mice have dramatically reduced lesions (~0.3% of control lesion size) but heterozygous (op/+) mice had lesions <1% of controls. Plasma M-CSF levels in heterozygous (op/+) mice were about half of those in controls (+/+). The finding that a ~2-fold reduction in M-CSF expression reduced lesion size ~100-fold suggests the requirement for a threshold level of M-CSF. The effects of M-CSF on atherosclerosis in LDLR^{-/-} mice did not appear to be mediated by changes in plasma lipoproteins or alterations in the number of circulating monocytes, since both op/op and op/+ mice exhibited higher levels of atherogenic lipoprotein particles,⁸⁶ and (op/+) mice showed a near normal number of circulating monocytes. Taken together, these results suggest that the effects of M-CSF on atherogenesis are not mediated by systemic M-CSF or by changes in monocyte numbers, but that local M-CSF plays an essential role in the arterial wall itself. Importantly, M-CSF is critical for promoting the early stages of atherosclerosis since administration of a monoclonal antibody against *c-fms*, the receptor of M-CSF, protects from early atherogenesis but does not attenuate progression of already developed lesions in ApoE^{-/-} mice.⁸⁷ SMC can also express *c-fms* and it has been suggested that activation of these cells by M-CSF may promote development of smooth-muscle-derived foam cells.

Similar to M-CSF, GM-CSF is induced in EC by oxidized lipids⁸⁴ and abundantly present in atherosclerotic lesions.⁸⁸ Its function overlaps with that of M-CSF. GM-CSF appears a major regulator specifically of dendritic cell differentiation and aortic lesional dendritic cell accumulation. So far, reported findings on the effect of GM-CSF on atherosclerosis are inconsistent. Hyperlipidaemic LDLR^{-/-} mice with a GM-CSF deficiency exhibited ~20–50% decrease in aortic lesion size, depending on the location of the lesions and the sex of the animals.⁸⁸ Similarly, treatment of male ApoE^{-/-} mice (fed a cholate-containing diet) with murine GM-CSF (10 µg kg⁻¹ day⁻¹ subcutaneously) increased the atherosclerotic lesion area by 2-fold.⁸⁹ In contrast to both studies, genetic deletion of GM-CSF in ApoE^{-/-} mice increased atherosclerotic lesion size, without affecting plasma cholesterol.⁹⁰ In the latter study, animals were not fed cholate. Furthermore, the collagen content in the lesions was reduced, suggesting the formation of unstable plaques.

While both M-CSF and, to some lesser extent, GM-CSF have been extensively studied in atherosclerosis and other inflammatory disorders, G-CSF and multi-CSF/IL-3 have received hardly any attention hitherto. Haghghat *et al.*⁸⁹ showed that subcutaneous administration of G-CSF (10 µg kg⁻¹ day⁻¹) in ApoE^{-/-} mice over an 8-week period resulted in a 44% increase in atherosclerotic lesion extent,

without alterations in plasma lipids or vascular and systemic inflammation markers. No studies on the role of IL-3 in atherosclerosis have been described yet.

In summary, of the four CSFs characterized, only endogenous M-CSF has been consistently found proatherogenic, most probably by influencing the lesional macrophage phenotype. GM-CSF has been attributed both pro-atherogenic and anti-atherogenic properties, while the role of G-CSF and multi-CSF/IL-3 in atherosclerosis needs further research.

16. Summary and conclusions

Inflammatory processes are involved at all stages of the atherosclerotic process, from lesion initiation to plaque rupture. Potentially, cytokines play an important role in the pathogenesis of atherosclerosis and thus could act as important targets for anti-atherosclerotic strategies, on top of those directed at cholesterol-lowering.

Here, we have summarized all currently available information from mice studies on the contribution of interleukins, macrophage-related cytokines, and CSFs to lesion evolution. *Table 2* provides a global overview on the overall effects of these cytokines on plasma cholesterol and atherosclerosis in C57/BL6, ApoE^{-/-}, and LDLR^{-/-} mice. An important outcome of this survey is that several cytokines show explicit and consistent pro-atherogenic effects, generally independent of the experimental approach and conditions chosen: IL-1, IL-12, IL-18, TNF-α, MIF, IFN-γ, and M-CSF all display pro-atherogenic characteristics while IL-10 clearly has anti-atherogenic properties (*Figure 2B*). Some cytokines have a dual character and show variable effects: IL-4, IL-6, and GM-CSF can have pro- or anti-atherogenic properties, depending on the mouse model and gender used, disease stage analysed, and dietary conditions applied. Only a few studies have tested IL-2, IL-20, G-CSF (all showing pro-atherogenic characteristics so far), or IL-5 (anti-atherogenic to this point), and additional experimental evidence is required before definitive conclusions can be drawn. Another key result of this review is that several of the pro-atherogenic cytokines (IL-1, IL-18, TNF-α, IFN-γ, MIF, and M-CSF) affect plasma cholesterol levels, underscoring the notion that inflammation and lipid metabolism are interlinked processes.^{2,91–93}

Overall, our survey provides support for the notion that modulating the activity of selected cytokines (either systemically or locally) can block or retard the development of atherosclerotic lesions and thus positively influence disease outcome. The significant impact of selected cytokines on atherogenesis could be of significance for early detection and prevention of the disease, and also provides new opportunities for anti-atherosclerotic treatment regimens, alone or in combination with established hypolipidaemic or hypotensive strategies. Importantly, in the studies described in this review, the activity of the cytokine of interest was modulated by techniques such as genetic deletion, overexpression, immunoneutralization, or *in vivo* administration of the cytokine or its receptor or inhibitor, techniques not easily applicable to humans. The chronic nature of the atherosclerotic disease process and the generally pleiotropic effects of cytokines will demand high specificity of action and/or effective

Table 2 Comparison of the overall effects of the cytokines reviewed on plasma cholesterol levels and atherosclerosis in C57BL6 mice, ApoE^{-/-} mice, and LDLr^{-/-} mice

Mouse model	Approach	C57BL6		ApoE ^{-/-}		LDLr ^{-/-}	
		Chol	Athero	Chol	Athero	Chol	Athero
IL-1	IL-1R antagonist (IL1Ra) exogenous	x	x	↔	↓	x	x
	tg IL-1Ra overexpression	x	x	↔	↓	↑ (↔ with cholate)	↔ (↓ with cholate)
	IL-1Ra ^{-/-}	↓	↑	↑	↑	x	x
	IL-1α ^{-/-}	↑ (↔ BM)	↓	x	x	x	x
	IL-1β ^{-/-}	↔	↓	↔	↓	x	x
IL-2	IL-1R	x	x	x	↓	x	x
	Exogenous	x	x	x	↑	x	x
IL-4	Anti-IL-2-AB	x	x	x	↓	x	x
	Exogenous	↔	↓	x	x	x	x
IL-5	-/-	↔	va	↔	↓	↔	↔
	-/-	x	x	x	x	x	↑
IL-6	Recombinant	↔	↑	↔	↑	x	x
	-/-	x	↑	va (↔↑)	va (↔↑)	↔	↔
IL-10	Transgenic	↔	↓	x	x	↔	↓
	-/-	↔	↑	↔	↑	↔	↑
IL-12	Gene transfer	x	x	va	↓	x	↓
	Exogenous	x	x	↔	↑	↔	↓
IL-18	p40 ^{-/-}	x	x	↔	↓	x	x
	Anti-IL-12-AB	x	x	x	x	x	↓
IL-18	Exogenous	x	x	↔	↑	x	x
	-/-	x	x	↑	↓	x	x
IL-20	IL-18 bpm	x	x	x	↓	x	x
	Exogenous	x	x	x	↑	x	x
TNF	TNFR ^{-/-}	↔	↑p55 ^{-/-} ↔p75 ^{-/-}	↔	↔↓ (stage-dependent)	x	x
	TNFα ^{-/-}	↑	↔↓	↔	↓	x	x
	tmTNF	↔	↓	x	x	x	x
	Anti-TNF	x	x	↔	↓	x	x
	TNFbp	x	x	↔	↓ (f)↔(m)	x	x
IFN-γ	IFNγ ^{-/-}	x	x	↔	↓	↔	↓
	IFNγR ^{-/-}	x	x	↑	↓	x	x
MIF	Exogenous	x	x	↔	↑	x	x
	Soluble IFNγR	x	x	↔	↓	x	x
M-CSF	-/-	x	x	x	x	↓	↓
	Anti-MIF-AB	x	x	↔	↓	↔	↓
GM-CSF	-/-	↑	↓	↑	↓	↑	↓
	Anti-M-CSF receptor	x	x	↔	↓	x	x
G-CSF	-/-	x	x	↔	↑	↔	↓
	Exogenous	x	x	↔	↑	x	x
G-CSF	Exogenous	x	x	↔	↑	x	x

x, no data available; ↑, increase; ↓, decrease; ↔, no effect; va, variable despite comparable experimental conditions; AB, antibody; BM, bone marrow transplantation; tm, transmembrane; f/m, female/male.

targeting to prevent the emergence of adverse side effects with clinical applications.⁹⁴

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