thematic review

Thematic review series: The Immune System and Atherogenesis

Cytokine regulation of macrophage functions in atherogenesis

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Abstract **This review will focus on the role of cytokines in the behavior of macrophages, a prominent cell type of atherosclerotic lesions. Once these macrophages have immigrated into the vessel wall, they propagate the development of atherosclerosis by modifying lipoproteins, accumulating intracellular lipids, remodeling the extracellular environment, and promoting local coagulation. The numerous cytokines that have been detected in atherosclerosis, combined with the expression of large numbers of cytokine receptors on macrophages, are consistent with this axis being an important contributor to lesion development. Given the vast literature on cytokine-macrophage interactions, this review will be selective, with an emphasis on the major cytokines that have been detected in atherosclerotic lesions and their effects on properties that are relevant to lesion formation and maturation. There will be an emphasis on the role of cytokines in regulating lipid metabolism by macrophages. We will provide an overview of the major findings in cell culture and then put these in the context of in vivo studies.**—Daugherty, A., N. R. Webb, D. L. Rateri, and V. L. King. **Cytokine regulation of macrophage functions in atherogenesis.** ***J. Lipid Res.*** **2005.** 46: **1812–1822.**

Supplementary key words lipoprotein • modification • metabolism • matrix • coagulation

As noted in Getz's overview (1), lesions contain large numbers of cytokines that can be derived from several cell types. These cytokines may affect the function of many cell types in atherogenesis. The effects of cytokines on endothelial and smooth muscle cells are discussed in Raines and Ferri's contribution to this series (2). The purpose of this review is to focus on the effects of cytokines on macrophages in the evolution of atherosclerotic lesions. This is a vast literature that has necessitated some selectivity in the areas that can be covered. Given the subject area of this journal, we have elected to focus particular attention on the effect of cytokines on lipid metabolism in macrophages.

MACROPHAGE FUNCTIONS IN ATHEROSCLEROTIC LESIONS

Macrophages are hypothesized to be attracted to the subendothelial space to remove noxious materials deposited at atherosclerosis-prone regions of arteries. The precise chemical identity of the substance that attracts macrophages has not been unequivocally defined, although many candidate molecules are components of modified lipoproteins (3, 4). However, the function of infiltrating cells becomes subverted and leads to their retention within the subendothelial space. In this region, it is proposed that macrophages modify adjacent lipoproteins while also providing major mechanisms of removal for modified materials from the extracellular environment. The combination of lipoprotein modification and uptake leads to macrophages becoming engorged with lipids and resulting in a morphology that is given the descriptive name of "foam cells." Lipid engorgement causes pronounced cellular hypertrophy, with the cell diameter being >10 times that of the originating monocyte. Probably as a result of the immense size increase, lipid-laden macrophages are chronically entrapped in the subendothelial space. Trapped macrophages can then invoke processes that perpetuate the continual recruitment of monocytes, leading to an expanded lesion volume. In addition, the subendothelial macrophages can influence the behavior of neighboring cell types within atherosclerotic lesions.

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Abbreviations: apoE, apolipoprotein E; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; LOX-1, lectin-like oxidized low density lipoprotein receptor-1; LRP, low density lipoprotein receptor-related protein; M-CSF, monocyte colony-stimulating factor; MMP, matrix metalloproteinase; SR-A, class A scavenger receptor; SR-BI, scavenger receptor class B type I; TGF, transforming growth factor; TNF, tumor necrosis factor.

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This includes the well-characterized interaction of macrophages and T-lymphocytes (5). During late stages of atherosclerosis development, exposure of macrophage-rich areas of lesions provides a nidus for thrombus attachment that is thought to account for a high proportion of the catastrophic consequences of atherosclerosis. At each stage of lesion development described above, cytokine interactions with macrophages have the potential to be major determinants of the mechanism and magnitude of the response.

EXPERIMENTAL MODES IN ELUCIDATING MACROPHAGE BIOLOGY IN ATHEROSCLEROSIS

The macrophage is probably the most phenotypically diverse cell type in the body (6). From the common origin of monocytes, macrophages take on different characteristics that are presumably determined by the local milieu of the tissue into which they migrate. Although dissimilar between tissues, macrophages within a specific tissue have less heterogeneity. However, this is not the case in atherosclerosis, in which there are considerable variances in the structures of macrophages that may reflect a divergent array of functional abilities.

Many contributions to our knowledge of cytokine effects on macrophage biology in atherosclerosis are derived from studies of cultured cells. The classic system in this area of investigation is primary cultures of macrophages derived from peritoneal lavage of mice (7). Many studies have also used macrophages derived from human monocytes. In addition to primary cells, there are also a wide variety of macrophage-like cell lines. Some of the most commonly used cell lines include the human-derived THP-1 (8) and the mouse lines J774 and P388D1 (9, 10). Although these cultured cells have some macrophage characteristics, the extent to which they mimic the cells present in atherosclerosis remains an open question. The assessment of similarities and differences of macrophages in culture versus those within atherosclerotic lesions is hampered by the fluid nature of macrophage phenotypes and the dearth of reagents to specifically define these phenotypes. The combination of this extensive heterogeneity and the imprecision of phenotyping may contribute to the conflicting reports of the effects of cytokines on some properties of macrophage biology that are described in this review.

CYTOKINE REGULATION OF MACROPHAGE-MEDIATED LIPOPROTEIN MODIFICATION

An early event in the development of atherosclerotic lesions is the subendothelial deposition of lipoproteins (11, 12). The presence of lipoproteins in the subendothelial space leads to substances that promote both chemotaxis and lipid accumulation in macrophages. Several modifications can promote both of these properties (3, 13). In the early phases of lesion formation, lipoprotein modification occurs, presumably mostly mediated by endothelial mechanisms (14). However, once atherogenesis has been initiated, macrophages may become a major cell type responsible for lipoprotein modification within lesions.

There have been several pathways proposed for lipoprotein modification that are regulated by interactions of cytokines with macrophages. Much of the focus of the study of these modifications has been on oxidative mechanisms. Oxidation of LDL by cultured mouse peritoneal macrophages, as defined by the content of thiobarbituric acid-reacting substances, is decreased by incubating cells with interferon (IFN)-γ (15). One potential mechanism of IFN-γ-induced reduction of LDL oxidation is an acceleration of extracellular tryptophan degradation (16). Several reports have demonstrated a role of IFN-γ via a lipoxygenase pathway that is also thought to involve oxidation (17). Lipoxygenase, specifically the 15-isoenzyme, is expressed in lesional macrophages and colocalizes with immunologically defined oxidative epitopes of lipoproteins (18, 19). Expression of the enzyme in macrophages has been shown to promote atherosclerosis and lipoprotein oxidation (20–22), although there are contrary data (23, 24). 15-Lipoxygenase is not expressed in cultured human monocytes, but its expression is profoundly stimulated by incubation with interleukin (IL)-4 or IL-13 (25, 26). The stimulation of lipoxygenase expression in these cells is ablated by coincubation with IFN-γ (25). However, incubation of IFN-γ with mouse peritoneal macrophages, which contain abundant lipoxygenase activity on isolation, does not influence lipoxygenase protein abundance or activity (27). Therefore, the effect of IFN-γ appears to be via a mechanism that inhibits the synthesis of the lipoxygenase. The contribution of these cytokines to lipoxygenase regulation in vivo is unclear, because mice deficient in IL-4 do not have reduced expression of lipoxygenase (27). This may be attributable to the continued presence of IL-13 (26). However, lipoxygenase expression is unexpectedly increased in total lymphocyte-deficient mice that are assumed to have low circulating concentrations of cytokines. Also, there is no effect of STAT-6 deficiency, which is a common pathway for the effects of both IL-4 and IL-13 (28).

Myeloperoxidase is present in large amounts in monocytes and has been proposed as a major oxidative enzyme in atherosclerosis (29). The role of myeloperoxidase in the development of atherosclerosis has not been defined, although the protein and many oxidative products of its activity have been detected in lesions (29–32). The cytokine regulation of myeloperoxidase in macrophages has been given limited attention. Granulocyte macrophage colony-stimulating factor (GM-CSF), but not monocyte colony-stimulating factor (M-CSF), increases the expression of myeloperoxidase in macrophages (33).

Lipoproteins can also be modified within lesions by nonoxidative processes. These include the effects of LPL expressed in macrophages of atherosclerotic lesions (34). The absence of LPL in macrophages decreases atherosclerosis (35, 36), whereas overexpression in this cell type increases lesion size (37). IFN-γ decreases the expression of

LPL in human monocyte-derived macrophages from early to late stages of culture (38, 39) and by transcriptional regulation (40). Macrophage LPL expression is also inhibited by transforming growth factor (TGF)-β through a transcriptional effect (41, 42), but it is upregulated by tumor necrosis factor (TNF)-α (43). Thus, there is substantial evidence that cytokine regulation may be an important contributor to the atherogenic effects of macrophage-expressed LPL. Although LPL can modify specific lipoprotein fractions, it is possible that nonlipolytic properties of the protein are responsible for its effects in atherogenesis (44).

CYTOKINE REGULATION OF LIPOPROTEIN ENTRY INTO MACROPHAGES

One of the most prominent changes in macrophages after entry into the subendothelial space of developing atherosclerotic lesions is the engorgement of these cells with lipid. Intracellular lipid stores are initially formed with simple droplet morphology. With progressive lipid engorgement, there is the formation of intracellular complexes of cholesterol and phospholipid and cholesterol crystals (45, 46). These complexes and crystals are frequently encased by an acid phosphatase-positive layer, consistent with entrapment in lysosomes or late endosomes.

It is now recognized that many receptors are present on macrophages that bind a wide range of native and modified lipoproteins. Several major receptor types that recognize native lipoproteins may be regulated by cytokines in macrophages. LDL receptors have a clearly defined role in the cholesterol homeostasis of the whole body. Their role in macrophages has not been explored widely because of the assumption that they are downregulated in lipid-laden lesional macrophages. However, LDL receptor protein is detectable in experimental atherosclerotic lesions (47, 48). Furthermore, macrophage LDL receptors influence atherogenesis under conditions of modest hyperlipidemia (48, 49). The expression of LDL receptors on macrophages in cultured cells is a function of the origin and differentiation status (50). Macrophage LDL receptors have been demonstrated to be upregulated and downregulated by IFN-γ and TGF-β, respectively (41, 51). Expression of LDL receptors may also have implications on the mode of metabolism of the highly atherogenic lipoprotein fraction, β-VLDL, which may have characteristics similar to those of postprandial chylomicron lipoproteins (48, 49).

In addition to LDL receptors, β-VLDL is also recognized by VLDL receptors. This receptor type is also expressed on macrophages and is downregulated by IFN-γ (52). The functional significance of this downregulation can be shown by the inhibition of β-VLDL-induced foam cell formation by IFN-γ.

Another lipoprotein receptor type that recognizes native lipoproteins is the low density lipoprotein receptor-related protein (LRP; also designated CD91) (53, 54). Although the embryonic lethality of LRP-deficient mice has impeded a full characterization of its function, its properties appear consistent with being the major system for the clearance of chylomicron remnants from the plasma (54). LRP on macrophages has consistently been shown to be downregulated in the presence of IFN-γ (55–57). Conversely, TGF-β upregulates macrophage LRP, whereas M-CSF has no effect (56). These changes in macrophage LRP expression may have implications for lipoprotein accumulation within lesional macrophages. LRP, which is also termed the α2 macroglobulin receptor, has a wide range of ligands in addition to lipoproteins (54). Many of these ligands are responsible for regulating the extracellular proteolytic environment of macrophages (58).

Although there has been limited work on the cytokine regulation of native lipoprotein receptors, there has been considerable effort to study the cytokine regulation of receptors for modified lipoproteins. As discussed in the preceding section, there are several mechanisms of lipoprotein modification that can be regulated by cytokines. Many of these modifications involve some form of oxidative damage. The original receptor for modified lipoproteins was designated a "scavenger receptor" based on its ability to mediate the endocytosis of acetylated LDL (59). There are now many proteins that have been designated as scavenger receptors that are broadly classified by gross structural characteristics in an alphabetic system (8).

The initially discovered scavenger receptor is now referred to as class A scavenger receptor (SR-A) (60). This receptor is able to transport acetylated and oxidized forms of LDL into macrophages by a process that is not downregulated by intracellular cholesterol content (61). Several studies have used genetically manipulated mice to define the effect of SR-A on atherosclerotic lesion formation (62). The ability of cytokines to regulate the SR-A receptor has been the subject of many publications, of which some selected examples are summarized in **Table 1**. IFN-γ is the most widely investigated cytokine. Earlier studies demonstrated a downregulation of SR-A by IFN-γ in human monocyte-derived macrophages (63, 64). IFN-γ has also been reported to inhibit SR-A at the transcriptional level (65). Decreased foam cell formation occurs by the incubation of IFN-γ with modified lipoprotein and macrophages (64). These effects, combined with studies on smooth muscle proliferation (66, 67), suggest that IFN-γ may reduce the atherogenic process. However, there are also reports of IFN-γ having other effects on SR-A activity, protein, and transcription that are either neutral or inhibitory (27, 68, 69). Markedly different results have also been reported for GM-CSF, which has shown both upregulation and downregulation of SR-A activity by this cytokine (27, 70). These differing responses may be a reflection of the heterogeneity of macrophages, which may lead to differences in the phenotype of the cultured cells. Consistent with this theme, IFN-γ increases SR-A expression in the early differentiation phase of blood-borne monocytes or the human cell line THP-1, but it downregulates this receptor in mature macrophages (69). These conflicting findings may also be related to the mode of data representation, because both IFN-γ and GM-CSF may markedly increase cellular protein content without influencing cell

TABLE 1. Cytokine regulation of macrophage lipoprotein receptors

Receptor	Cytokine	Effect on Receptor Abundance	Reference
Receptors facilitating transport of native lipoproteins into macrophages			
LDL receptor	IFN-γ	↑	51
	TGF-β	↓	41
VLDL receptor	IFN-γ	↓	52
LRP	IFN-γ	↓	55–57
	TGF-β	↑	56
	M-CSF	↔	55
Receptors facilitating transport of modified lipoproteins into macrophages			
SR-A	IFN-γ	↑	27, 69
		↓	63–65, 69
		↔	68
	TNF-α	↓	71, 74
	TGF-β	↓	148–150
	IL-4	↑	27
	IL-6	↓	151
	GM-CSF	↓	70
		↑	27,65
	M-CSF	↑	152, 153
CD36	IFN-γ	↔	81
		↓	82, 83
	TGF-β1	↓	84, 85
	IL-4	↑	80, 81
	M-CSF	↑	81
LOX-1	TGF-β	↑	154
		↓	148
	TNF-α	↑	88, 89
	IL-4	↑	155
CXCL16/SR-PSOX	IFN-γ	↑, associated with increased oxidized LDL uptake by THP-1 cells	156
Receptors that facilitate both lipid entry and efflux in macrophages			
SR-BI	IFN-γ	↓	96
	TNF-α	↓	96
	TGF-β1	↓	85

GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; LOX-1, lectin-like oxidized low density lipoprotein receptor-1; LRP low density lipoprotein receptor-related protein; M-CSF, monocyte colony-stimulating factor; SR-A, class A scavenger receptor; SR-BI, scavenger receptor class B type I; SR-PSOX, scavenger receptor that binds phosphatidylserine and oxidized lipoprotein; TGF, transforming growth factor; TNF, tumor necrosis factor.

number. Under these conditions, the normalization of data to either cellular protein or cell number can dramatically influence the conclusion of the study.

Lipopolysaccharide downregulates SR-A both in cultured cells and in vivo (71, 72), although induction has also been shown in both primary macrophage cells and mouse macrophage-like cell lines (73). Of the many cytokines released after the incubation of macrophages with lipopolysaccharide, the downregulation of SR-A has been attributed to the secretion of TNF-α (71). Consistent with this effect, SR-A is downregulated by incubation of cultured macrophages with TNF-α (74). Furthermore, deficiency of the TNF-α receptor, p55, has been associated with increased SR-A expression in atherosclerotic lesions (75). Some of the inconsistencies in SR-A expression during TNF-α incubation may be attributable to temporal considerations. Indeed, SR-A expression in macrophages has been shown to be increased before the subsequent inhibition (76).

In addition to the extensive literature on the effects of IFN-γ, CSFs, and TNF-α on SR-A regulation, there are a smaller number of publications that have studied the effects of other cytokines on this receptor. These are also summarized in Table 1.

Compared with SR-A, there is a relatively modest literature on the role of cytokines in regulating other scavenger receptors. CD36 is a receptor that has a number of diverse properties, including the binding of modified forms of lipoproteins, although its ability to facilitate endocytosis of these particles is not universally accepted (77). CD36 deficiency has been shown to decrease the extent of atherosclerosis, primarily through a macrophage-mediated effect (78, 79). The expression of CD36 on macrophages is increased by IL-4 and M-CSF (80, 81). The effects of IFN-γ and TGF-β on CD36 expression in macrophages have been inconsistent (81–85).

The lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) was originally identified in endothelium and subsequently shown to be expressed in macrophages (86, 87). Much of the focus of the study of its regulation has been on endothelial cells. In macrophages, variable effects of TGF-β1 on LOX-1 regulation have been reported, although TNF-α has consistently been shown to upregulate this receptor (88–90).

Scavenger receptor class B type I (SR-BI) was originally identified as a protein that binds oxidized LDL before the realization that it is a physiologically important HDL receptor (91, 92). SR-BI is expressed in macrophages and is distinct from the other receptors discussed above in that it can transfer lipid bidirectionally (8). The presence of SR-BI in macrophages has important consequences on the development of atherosclerosis, although the effect is complex, as may be expected for a receptor with this duality of function (93, 94). SR-BI expression is variable in macrophages present in atherosclerotic lesions (95). The mechanisms responsible for this variable SR-BI expression have not been determined. In the scant cytokine literature, it has been demonstrated that both IFN-γ and TNF-α downregulate SR-BI (96).

Overall, there are many receptors that facilitate the entry of native and modified lipoproteins into macrophages by transfer of either the whole particle or selective components. Although there has been some consistency in the effects of selected cytokines in regulating some of these receptors on macrophages, there are also many inconsistencies in the literature. Resolution of these conflicting results needs to account for the many variables in these studies, such as macrophage phenotype and temporal effects.

CYTOKINE REGULATION OF INTRACELLULAR LIPID DISTRIBUTION AND REMOVAL

The previous section described processes that predominantly influence the mass of cholesterol transported into the intracellular compartment. The mode in which cholesterol is distributed within macrophages has dramatic effects on its toxicity and availability for efflux. With regard to toxicity, unesterified cholesterol appears to be benign when excess is stored in lysosomes, but it has profound effects on cell function and viability when present in high concentrations in the cell membrane (97, 98). Efflux of cholesterol is dependent on several known variables, including the efficiency of cholesteryl ester hydrolysis and transport pathways involved in shuttling cholesterol to the cell surface and its subsequent removal.

In addition to its role in macrophage-mediated lipoprotein modification and uptake, IFN-γ has also been extensively studied with regard to its effects on macrophage lipid metabolism (**Table 2**). IFN-γ is the only cytokine in which a change in intracellular cholesterol storage has been demonstrated, with an increased cholesteryl ester pool and decreased cholesterol efflux (82, 99). This increase could be accomplished by increased activity of ACAT-1, which esterifies cholesterol to cholesteryl ester. The activity of this enzyme is increased in macrophages incubated with IFN-γ (100). M-CSF and TGF-β1 also increase ACAT-1 activity (101, 102). The effect of these cytokines on intracellular cholesterol distribution has not been determined.

The removal of cholesterol from the cell requires the hydrolysis of cholesteryl ester, which is achieved by either acid or neutral forms of cholesteryl ester hydrolase. The cytokine regulation of these enzymes is not well studied, with only one report demonstrating the increased activity of both forms of cholesteryl ester hydrolase after incubation of macrophages with M-CSF (102). Another cholesterol removal process is via the activity of cholesterol 27-hydroxylase, which is known to be present in many extrahepatic cells, including macrophages (103). Cholesterol 27-hydroxylase converts cholesterol to more polar products that are readily removed from macrophages. This process is upregulated by IFN-γ (104, 105).

The secretion of cholesterol from macrophages is influenced by the ability to secrete apolipoprotein E (apoE), which has been demonstrated to promote cholesterol efflux, particularly when endogenously produced (106–108). IFN-γ decreases apoE secretion via a posttranslational mechanism (109). Several other cytokines have also been demonstrated to regulate apoE secretion, although these have not been linked to changes in cholesterol efflux (Table 2).

ABCA1 has a well-defined role in cholesterol efflux in many cell types, including macrophages (110). The cytokine regulation of ABCA1 in macrophages is consistent with its role in efflux. For example, decreased abundance after IFN-γ incubation was associated with decreased HDL binding and cholesterol efflux. This effect was independent of changes in the expression of SR-BI (111, 112).

TABLE 2. Cytokine regulation of intracellular lipid metabolism in macrophages

Effect	Cytokine	Effect	Reference
Cholesterol distribution	IFN-γ	↑ in cholesteryl esters	82
ACAT-1	IFN-γ	↑	100
	TGF-β1	↑	101
	M-CSF	↑	102
Cholesteryl ester hydrolases	M-CSF	↑	102
Cholesterol 27-hydroxylase	IFN-γ	↑	99, 104
Apolipoprotein E secretion	IFN-γ	↓ secretion, due to posttranslational effect	109, 157
	IL-1	↓ synthesis	109
	GM-CSF	↓ synthesis	109
	TNF-α	↑ (only in monocyte, not macrophages)	158
	TGF-β	↑ secretion	109
ABCA1	IFN-γ	↓ expression, with Ø in cholesterol efflux	111, 112
	TGF-β	↑ expression, with ≠ cholesterol efflux	41, 113
ABCG1	TGF-β	↑	41
HDL binding	IFN-γ	↓, but in absence of effects on SR-BI	111
	TGF-β	↓	113

Conversely, TGF-β1 increased ABCA1 expression and cholesterol efflux (41, 105, 113), although the binding of HDL was decreased by this cytokine (85).

Cytokines have many effects on macrophages with regard to intracellular cholesterol metabolism and its consequences on sterol efflux. With few anomalies, the most extensively studied cytokine, IFN-γ, increases intracellular cholesterol storage when cells are not incubated with modified lipoproteins. Further studies to demonstrate whether these changes affect macrophage viability would be of interest (98).

CYTOKINE REGULATION OF THE EXTRACELLULAR MATRIX OF MACROPHAGES

The extracellular matrix of the artery contains many different proteins. The subendothelial region contains type IV collagen, laminin, and fibronectin. The integrity of extracellular matrix proteins in atherosclerotic lesions may be compromised by a large number of enzymes, most of which belong to the matrix metalloproteinease (MMP), cysteine protease, or serine protease families.

Many MMPs have been detected in atherosclerosis, although the dominant MMPs expressed by macrophages that have been implicated in lesion development are MMP-9 and MMP-12. Of these MMPs, only MMP-9 has been shown to alter the atherogenic process with its deficiency, reducing lesion size in several vascular areas (114). Moreover, this study demonstrated that bone marrow-derived stem cells were a significant source of the MMP-9 that modulates atherogenesis.

The Th2 cytokines IL-4 and IL-10 attenuated MMP-9 expression and activation in peripheral blood monocytes, mouse peritoneal macrophages, and human alveolar macrophages (115–117). Activation of MMP-9 is tightly regulated by tissue inhibitor of MMP-1, which is markedly upregulated by IL-10 (117, 118). TGF-β decreases lipopolysaccharide-induced MMP-9 expression and activation in cultured MM6 and RAW 264.7 cells (119). Conversely, TNF-α upregulated MMP-9 in human peripheral blood monocytes and peritoneal macrophages (120, 121). However, the effect of IFN-γ on the regulation of MMP-9 is unclear, with some studies demonstrating that it upregulates MMP-9 in mouse peritoneal macrophages and others suggesting that it downregulates MMP-9 (122–125).

CYTOKINE REGULATION OF COAGULATION IN ATHEROSCLEROSIS

The presence of tissue factor in atherosclerotic lesions is proposed to exert a major effect on the development of atherosclerosis complications at late stages of the disease (126). Tissue factor is expressed in all of the major cell types of atherosclerotic lesions, with a preponderance in macrophages (127, 128). Tissue factor expression in cultured macrophages is downregulated by the Th2 cytokines IL-4, IL-10, and IL-13, whereas the Th1 cytokine IFN-γ has the opposite effect (129, 130). Some of the effects on tissue factor expression, particularly those of IFN-γ, are differentiation-specific (131, 132). Overall, there is limited information regarding the role of cytokine-macrophage interactions on the effect of coagulation responses in atherosclerotic lesions.

EXTRAPOLATION OF CYTOKINE EFFECTS ON CULTURED CELLS TO THE ATHEROGENIC PROCESS IN VIVO

There have been numerous studies to determine the role of specific cytokines in the development of atherosclerosis. As described above, one cytokine that has been studied extensively in cell culture studies is IFN-γ, which is also one of the more extensively investigated cytokines in in vivo studies of atherogenesis. Studies with cultured cells have demonstrated many effects of IFN-γ on the intracellular accumulation of lipids in macrophages. These findings lead to the notion that IFN-γ would retard atherosclerosis, especially by minimizing intracellular lipid accumulation in macrophages. However, there are conflicting results in cultured cells. In contrast, the effects of IFN-γ on the development of atherosclerosis in mouse models of the disease have been quite consistent, but they have contradicted the original concept of IFN-γ being antiatherogenic. Thus, deletion of both IFN-γ and its receptor decreased the size of atherosclerotic lesions in apoE-deficient mice (133, 134). Deletion of IFN-γ also decreased the size of atherosclerotic lesions in LDL receptor-deficient mice (135). Conversely, exogenous administration of IFN-γ or its upstream regulators, IL-12 or IL-18, increased the extent of atherosclerosis (136–138). Currently, the only published report suggesting an antiatherogenic role for IFN-γ comes from a study of irradiated LDL receptor-deficient mice that were repopulated with cells from IFN-γ-deficient mice. Under these conditions, the absence of IFN-γ in bone marrow-derived stem cells increased the size of lesions in three different vascular regions (139).

Although gene manipulation and exogenous cytokine administration have provided valuable insight into the atherogenic process, these do not localize the effect to a specific action of the cytokine on macrophages. One mode of focusing on cytokine-macrophage interactions is to use mice that are deficient in specific cytokine receptors. Previous studies have used the technique of bone marrow transplantation as a mode of determining a leukocyte-specific role in atherosclerosis (140, 141). This approach has been used in the chemokine field, for example, in studies using CCR2 and CXCR-2 (142, 143). However, bone marrow transplantation studies with cytokine receptor-deficient mice have not been performed to date. Although such studies provide valuable insight, their interpretation should be tempered by the potential for bone marrow-derived stem cells to differentiate beyond myeloid and lymphoid lineages (144, 145). Thus, the definition of an interaction of a macrophage with a specific

cytokine as a functionally significant event in atherogenesis will be facilitated by the ability to specifically regulate cytokine receptors in this cell type.

CONCLUSIONS

The macrophage is a pivotal cell type throughout the initiation and maturation of atherosclerotic lesions. The combination of the many cytokines present in atherosclerotic lesions and the abundant cytokine receptors on macrophages is consistent with an important role of cytokine-macrophage interactions in lesion development. However, the abundance of both cytokines and receptors on macrophages also provides some challenges to unequivocally determining the relative importance and mechanism of a specific cytokine. The most obvious road ahead is the use of molecular engineering to enable the ablation and enhancement of cytokine responses in macrophages. With the increasing identification of specific cytokine receptors, coupled with evolving modes of altering gene expression in macrophages (146, 147), it is likely that this will spawn many studies to elucidate the role of cytokine interactions with macrophages.

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REFERENCES

1. Getz, G. S. 2005. Thematic review series: the immune system and atherogenesis. Immune function in atherogenesis. *J. Lipid Res.* **46:** 1–10.
2. Raines, E. W., and N. Ferri. 2005. Cytokines affecting endothelial and smooth muscle cells in vascular diseases. *J. Lipid Res.* **46:** 829–838.
3. Steinberg, D. 1997. Low density lipoprotein oxidation and its pathobiological significance. *J. Biol. Chem.* **272:** 20963–20966.
4. Shishehbor, M. H., and S. L. Hazen. 2004. Inflammatory and oxidative markers in atherosclerosis: relationship to outcome. *Curr. Atheroscler. Rep.* **6:** 243–250.
5. Hansson, G. K. 2001. Immune mechanisms in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1876–1890.
6. Gordon, S. 1995. The macrophage. *Bioessays.* **17:** 977–986.
7. Goldstein, J. L., Y. K. Ho, and M. S. Brown. 1980. Cholesteryl ester accumulation in macrophages resulting from receptor-mediated uptake and degradation of hypercholesterolemic canine beta-very low density lipoproteins. *J. Biol. Chem.* **255:** 1839–1848.
8. Du, B. H., C. Z. Fu, K. C. Kent, H. Bush, A. H. Schulick, K. Kreiger, T. Collins, and T. A. McCaffrey. 2000. Elevated Egr-1 in human atherosclerotic cells transcriptionally represses the transforming growth factor-beta type II receptor. *J. Biol. Chem.* **275:** 39039–39047.
9. Okwu, A. K., X. X. Xu, Y. Shiratori, and I. Tabas. 1994. Regulation of the threshold for lipoprotein-induced acyl-CoA:cholesterol O-acyltransferase stimulation in macrophages by cellular sphingomyelin content. *J. Lipid Res.* **35:** 644–655.
10. Daugherty, A., S. C. Whitman, A. E. Block, and D. L. Rateri. 2000. Polymorphism of class A scavenger receptors in C57BL/6 mice. *J. Lipid Res.* **41:** 1568–1577.
11. Kruth, H. S. 1985. Subendothelial accumulation of unesterified cholesterol. An early event in atherosclerotic lesion development. *Atherosclerosis.* **57:** 337–341.
12. Frank, J. S., and A. M. Fogelman. 1989. Ultrastructure of the intima in WHHL and cholesterol-fed rabbit aortas prepared by ultra-rapid freezing and freeze etching. *J. Lipid Res.* **30:** 967–978.
13. Quinn, M. T., S. Parthasarathy, L. G. Fong, and D. Steinberg. 1987. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc. Natl. Acad. Sci. USA.* **84:** 2995–2998.
14. Williams, K. J., and I. Tabas. 1995. The response-to-retention hypothesis of early atherogenesis. *Arterioscler. Thromb. Vasc. Biol.* **15:** 551–561.
15. Fong, L. G., T. S. E. Albert, and S. E. Hom. 1994. Inhibition of the macrophage-induced oxidation of low density lipoprotein by interferon-gamma. *J. Lipid Res.* **35:** 893–904.
16. Christen, S., S. R. Thomas, B. Garner, and R. Stocker. 1994. Inhibition by interferon-gamma of human mononuclear cell-mediated low density lipoprotein oxidation—participation of tryptophan metabolism along the kynurenine pathway. *J. Clin. Invest.* **93:** 2149–2158.
17. Sun, D. X., and C. D. Funk. 1996. Disruption of 12/15-lipoxygenase expression in peritoneal macrophages—enhanced utilization of the 5-lipoxygenase pathway and diminished oxidation of low density lipoprotein. *J. Biol. Chem.* **271:** 24055–24062.
18. Ylä-Herttuala, S., M. E. Rosenfeld, S. Parthasarathy, C. K. Glass, E. Sigal, J. L. Witztum, and D. Steinberg. 1990. Colocalization of 15-lipoxygenase messenger RNA and protein with epitopes of oxidized low density lipoprotein in macrophage-rich areas of atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA.* **87:** 6959–6963.
19. Ylä-Herttuala, S., M. E. Rosenfeld, S. Parthasarathy, E. Sigal, T. Sarkioja, J. L. Witztum, and D. Steinberg. 1991. Gene expression in macrophage-rich human atherosclerotic lesions—15-lipoxygenase and acetyl low density lipoprotein receptor messenger RNA colocalize with oxidation specific lipid-protein adducts. *J. Clin. Invest.* **87:** 1146–1152.
20. Sendobry, S. M., J. A. Cornicelli, K. Welch, T. Bocan, B. Tait, B. K. Trivedi, N. Colbry, R. D. Dyer, S. J. Feinmark, and A. Daugherty. 1997. Attenuation of diet-induced atherosclerosis in rabbits with a highly selective 15-lipoxygenase inhibitor lacking significant antioxidant properties. *Br. J. Pharmacol.* **120:** 1199–1206.
21. Cyrus, T., J. L. Witztum, D. J. Rader, R. Tangirala, S. Fazio, M. F. Linton, and C. D. Funk. 1999. Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. *J. Clin. Invest.* **103:** 1597–1604.
22. Zhao, L., C. A. Cuff, E. Moss, U. Wille, T. Cyrus, E. A. Klein, D. Pratico, D. J. Rader, C. A. Hunter, E. Pure, et al. 2002. Selective interleukin-12 synthesis defect in 12/15-lipoxygenase-deficient macrophages associated with reduced atherosclerosis in a mouse model of familial hypercholesterolemia. *J. Biol. Chem.* **277:** 35350–35356.
23. Shen, J. H., E. Herderick, J. F. Cornhill, E. Zsigmond, H. S. Kim, H. Kuhn, N. V. Guevara, and L. Chan. 1996. Macrophage-mediated 15-lipoxygenase expression protects against atherosclerosis development. *J. Clin. Invest.* **98:** 2201–2208.
24. Belkner, J., P. Chaitidis, H. Stender, C. Gerth, R. J. Kuban, T. Yoshimoto, and H. Kuhn. 2005. Expression of 12/15-lipoxygenase attenuates intracellular lipid deposition during in vitro foam cell formation. *Arterioscler. Thromb. Vasc. Biol.* **25:** 797–802.
25. Conrad, D. J., H. Kuhn, M. Mulkins, E. Highland, and E. Sigal. 1992. Specific inflammatory cytokines regulate the expression of human monocyte 15-lipoxygenase. *Proc. Natl. Acad. Sci. USA.* **89:** 217–221.
26. Nassar, G. M., J. D. Morrow, L. J. Roberts, F. G. Lakkis, and K. F. Badr. 1994. Induction of 15-lipoxygenase by interleukin-13 in human blood monocytes. *J. Biol. Chem.* **269:** 27631–27634.
27. Cornicelli, J. A., D. Butteiger, D. L. Rateri, K. Welch, and A. Daugherty. 2000. Interleukin-4 augments acetylated LDL induced cholesterol esterification in macrophages. *J. Lipid Res.* **41:** 376–383.
28. Sendobry, S. M., J. A. Cornicelli, K. Welch, M. J. Grusby, and A. Daugherty. 1998. Absence of T lymphocyte-derived cytokines fails to diminish macrophage 12/15-lipoxygenase expression in vivo. *J. Immunol.* **161:** 1477–1482.
29. Daugherty, A., J. L. Dunn, D. L. Rateri, and J. W. Heinecke. 1994. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J. Clin. Invest.* **94:** 437–444.
30. Brennan, M. L., M. M. Anderson, D. M. Shih, X. D. Qu, X. Wang, A. C. Mehta, L. L. Lim, W. Shi, S. L. Hazen, J. S. Jacob, et al. 2001. Increased atherosclerosis in myeloperoxidase-deficient mice. *J. Clin. Invest.* **107:** 419–440.

31. Podrez, E. A., H. M. Abu-Soud, and S. L. Hazen. 2000. Myeloperoxidase-generated oxidants and atherosclerosis. *Free Radic. Biol. Med.* **28:** 1717–1725.
32. Heinecke, J. W. 2003. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. *Am. J. Cardiol.* **91:** 12A–16A.
33. Sugiyama, S., Y. Okada, G. K. Sukhova, R. Virmani, J. W. Heinecke, and P. Libby. 2001. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. *Am. J. Pathol.* **158:** 879–891.
34. Ylä-Herttuala, S., B. A. Lipton, M. E. Rosenfeld, I. J. Goldberg, D. Steinberg, and J. L. Witztum. 1991. Macrophages and smooth muscle cells express lipoprotein lipase in human and rabbit atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA.* **88:** 10143–10147.
35. Babaev, V. R., S. Fazio, L. A. Gleaves, K. J. Carter, C. F. Semenkovich, and M. F. Linton. 1999. Macrophage lipoprotein lipase promotes foam cell formation and atherosclerosis in vivo. *J. Clin. Invest.* **103:** 1697–1705.
36. van Eck, M., R. Zimmermann, P. H. Groot, R. Zechner, and T. J. van Berkel. 2000. Role of macrophage-derived lipoprotein lipase in lipoprotein metabolism and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **20:** E53–E62.
37. Wilson, K., G. L. Fry, D. A. Chappell, C. D. Sigmund, and J. D. Medh. 2001. Macrophage-specific expression of human lipoprotein lipase accelerates atherosclerosis in transgenic apolipoprotein E knockout mice but not in C57BL/6 mice. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1809–1815.
38. Jonasson, L., G. K. Hansson, G. Bondjers, L. Noe, and J. Etienne. 1990. Interferon-gamma inhibits lipoprotein lipase in human monocyte-derived macrophages. *Biochim. Biophys. Acta.* **1053:** 43–48.
39. Querfeld, U., J. M. Ong, J. Prehn, J. Carty, B. Saffari, S. C. Jordan, and P. A. Kern. 1990. Effects of cytokines on the production of lipoprotein lipase in cultured human macrophages. *J. Lipid Res.* **31:** 1379–1386.
40. Hughes, T. R., T. S. Tengku Muhammad, S. A. Irvine, and D. P. Ramji. 2002. A novel role of Sp1 and Sp3 in the interferon-gamma-mediated suppression of macrophage lipoprotein lipase gene transcription. *J. Biol. Chem.* **277:** 11097–11106.
41. Argmann, C. A., C. H. van den Diepstraten, C. G. Sawyez, J. Y. Edwards, R. A. Hegele, B. M. Wolfe, and M. W. Huff. 2001. Transforming growth factor-beta 1 inhibits macrophage cholesteryl ester accumulation induced by native and oxidized VLDL remnants. *Arterioscler. Thromb. Vasc. Biol.* **21:** 2011–2018.
42. Irvine, S. A., P. Foka, S. A. Rogers, J. R. Mead, and D. P. Ramji. 2005. A critical role for the Sp1-binding sites in the transforming growth factor-beta-mediated inhibition of lipoprotein lipase gene expression in macrophages. *Nucleic Acids Res.* **33:** 1423–1434.
43. Renier, G., E. Skamene, J. B. DeSanctis, and D. Radzioch. 1994. Induction of tumor necrosis factor alpha gene expression by lipoprotein lipase. *J. Lipid Res.* **35:** 271–278.
44. Williams, K. J., G. M. Fless, K. A. Petrie, M. L. Snyder, R. W. Brocia, and T. L. Swenson. 1992. Mechanisms by which lipoprotein lipase alters cellular metabolism of lipoprotein(a), low density lipoprotein, and nascent lipoproteins—roles for low density lipoprotein receptors and heparan sulfate proteoglycans. *J. Biol. Chem.* **267:** 13284–13292.
45. Jerome, W. G., and J. C. Lewis. 1997. Cellular dynamics in early atherosclerotic lesion progression in white carneau pigeons—spatial and temporal analysis of monocyte and smooth muscle invasion of the intima. *Arterioscler. Thromb. Vasc. Biol.* **17:** 654–664.
46. Jerome, W. G., and P. G. Yancey. 2003. The role of microscopy in understanding atherosclerotic lysosomal lipid metabolism. *Microsc. Microanal.* **9:** 54–67.
47. Boisvert, W. A., J. Spangenberg, and L. K. Curtiss. 1997. Role of leukocyte-specific LDL receptors on plasma lipoprotein cholesterol and atherosclerosis in mice. *Arterioscler. Thromb. Vasc. Biol.* **17:** 340–347.
48. Herijgers, N., M. van Eck, P. H. E. Groot, P. M. Hoogerbrugge, and T. J. C. van Berkel. 2000. Low density lipoprotein receptor of macrophages facilitates atherosclerotic lesion formation in C57B1/6 mice. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1961–1967.
49. Linton, M. F., V. R. Babaev, L. A. Gleaves, and S. Fazio. 1999. A direct role for the macrophage low density lipoprotein receptor in atherosclerotic lesion formation. *J. Biol. Chem.* **274:** 19204–19210.
50. Soutar, A. K., and B. L. Knight. 1984. Degradation of lipoproteins by human monocyte-derived macrophages. *Biochem. J.* **218:** 101–111.
51. Whitman, S. C., C. A. Argmann, C. G. Sawyez, D. B. Miller, R. A. Hegele, and M. W. Huff. 1999. Uptake of type IV hypertriglyceridemic VLDL by cultured macrophages is enhanced by interferon-gamma. *J. Lipid Res.* **40:** 1017–1028.
52. Kosaka, S., S. Takahashi, K. Masamura, H. Kanehara, J. Sakai, G. Tohda, E. Okada, K. Oida, T. Iwasaki, H. Hattori, et al. 2001. Evidence of macrophage foam cell formation by very low-density lipoprotein receptor: interferon-gamma inhibition of very low-density lipoprotein receptor expression and foam cell formation in macrophages. *Circulation.* **103:** 1142–1147.
53. Beisiegel, U., W. Weber, G. Ihrke, J. Herz, and K. K. Stanley. 1989. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature.* **341:** 162–164.
54. Herz, J., and D. K. Strickland. 2001. LRP: a multifunctional scavenger and signaling receptor. *J. Clin. Invest.* **108:** 779–784.
55. LaMarre, J., B. B. Wolf, E. L. W. Kittler, P. J. Quesenberry, and S. L. Gonias. 1993. Regulation of macrophage alpha2-macroglobulin receptor/low density lipoprotein receptor-related protein by lipopolysaccharide and interferon-gamma. *J. Clin. Invest.* **91:** 1219–1224.
56. Hussaini, I. M., J. LaMarre, J. J. Lysiak, L. R. Karns, S. R. VandenBerg, and S. L. Gonias. 1996. Transcriptional regulation of LDL receptor-related protein by IFN-gamma and the antagonistic activity of TGF-beta 1 in the RAW 264.7 macrophage-like cell line. *J. Leukoc. Biol.* **59:** 733–739.
57. Garner, B., A. Baoutina, R. T. Dean, and W. Jessup. 1997. Regulation of serum-induced lipid accumulation in human monocyte-derived macrophages by interferon-gamma. Correlations with apolipoprotein E production, lipoprotein lipase activity and LDL receptor-related protein expression. *Atherosclerosis.* **128:** 47–58.
58. Gonias, S. L., L. Wu, and A. M. Salicioni. 2004. Low density lipoprotein receptor-related protein: regulation of the plasma membrane proteome. *Thromb. Haemost.* **91:** 1056–1064.
59. Brown, M. S., S. K. Basu, J. R. Falck, Y. K. Ho, and J. L. Goldstein. 1980. The scavenger cell pathway for lipoprotein degradation: specificity of the binding site that mediates the uptake of negatively-charged LDL by macrophages. *J. Supramol. Struct.* **13:** 67–81.
60. Krieger, M., S. Acton, J. Ashkenas, A. Pearson, M. Penman, and D. Resnick. 1993. Molecular flypaper, host defense, and atherosclerosis—structure, binding properties, and functions of macrophage scavenger receptors. *J. Biol. Chem.* **268:** 4569–4572.
61. de Winther, M. P., K. W. van Dijk, L. M. Havekes, and M. H. Hofker. 2000. Macrophage scavenger receptor class A: a multifunctional receptor in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **20:** 290–297.
62. Daugherty, A., D. L. Rateri, and S. C. Whitman. 2000. Class A scavenger receptors: recent advances in elucidation of structure-function relationships and their role in atherosclerosis. *Curr. Opin. Cardiovasc. Pulm. Ren. Investig. Drugs.* **2:** 223–232.
63. Fong, L. G., T. A. T. Fong, and A. D. Cooper. 1990. Inhibition of mouse macrophage degradation of acetyl low density lipoprotein by interferon-gamma. *J. Biol. Chem.* **265:** 11751–11760.
64. Geng, Y. J., and G. K. Hansson. 1992. Interferon-gamma inhibits scavenger receptor expression and foam cell formation in human monocyte-derived macrophages. *J. Clin. Invest.* **89:** 1322–1330.
65. Horvai, A. E., L. Xu, E. Korzus, G. Brard, D. Kalafus, T. M. Mullen, D. W. Rose, M. G. Rosenfeld, and C. K. Glass. 1997. Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and p300. *Proc. Natl. Acad. Sci. USA.* **94:** 1074–1079.
66. Hansson, G. K., L. Jonasson, J. Holm, M. M. Clowes, and A. W. Clowes. 1988. Gamma-interferon regulates vascular smooth muscle proliferation and Ia antigen expression in vivo and in vitro. *Circ. Res.* **63:** 712–719.
67. Geng, Y. J., Q. Wu, M. Muszynski, G. K. Hansson, and P. Libby. 1996. Apoptosis of vascular smooth muscle cells induced by in vitro stimulation with interferon-gamma, tumor necrosis factor-alpha, and interleukin-1 beta. *Arterioscler. Thromb. Vasc. Biol.* **16:** 19–27.
68. de Whalley, C. V., and D. W. H. Riches. 1991. Influence of the cytocidal macrophage phenotype on the degradation of acetylated low density lipoproteins—dual regulation of scavenger receptor activity and of intracellular degradation of endocytosed ligand. *Exp. Cell Res.* **192:** 460–468.
69. Grewal, T., E. Priceputu, J. Davignon, and L. Bernier. 2001. Identification of a gamma-interferon-responsive element in the pro-

moter of the human macrophage scavenger receptor A gene. *Arterioscler. Thromb. Vasc. Biol.* **21:** 825–831.
70. Van der Kooij, M. A., O. H. Morand, H. J. Kempen, and T. J. C. van Berkel. 1996. Decrease in scavenger receptor expression in human monocyte-derived macrophages treated with granulocyte macrophage colony-stimulating factor. *Arterioscler. Thromb. Vasc. Biol.* **16:** 106–114.
71. van Lenten, B. J., and A. M. Fogelman. 1992. Lipopolysaccharide-induced inhibition of scavenger receptor expression in human monocyte-macrophages is mediated through tumor necrosis factor-alpha. *J. Immunol.* **148:** 112–116.
72. Roselaar, S. E., and A. Daugherty. 1997. Lipopolysaccharide decreases scavenger receptor mRNA in vivo. *J. Interferon Cytokine Res.* **17:** 573–579.
73. Fitzgerald, M. L., K. J. Moore, M. W. Freeman, and G. L. Reed. 2000. Lipopolysaccharide induces scavenger receptor A expression in mouse macrophages: a divergent response relative to human THP-1 monocyte/macrophages. *J. Immunol.* **164:** 2692–2700.
74. Hsu, H. Y., A. C. Nicholson, and D. P. Hajjar. 1996. Inhibition of macrophage scavenger receptor activity by tumor necrosis factor-alpha is transcriptionally and post-transcriptionally regulated. *J. Biol. Chem.* **271:** 7767–7773.
75. Schreyer, S. A., J. J. Peschon, and R. C. LeBoeuf. 1996. Accelerated atherosclerosis in mice lacking tumor necrosis factor receptor p55. *J. Biol. Chem.* **271:** 26174–26178.
76. Hsu, H. Y., and Y. C. Twu. 2000. Tumor necrosis factor-alpha-mediated protein kinases in regulation of scavenger receptor and foam cell formation on macrophage. *J. Biol. Chem.* **275:** 41035–41048.
77. Endemann, G., L. W. Stanton, K. S. Madden, C. M. Bryant, R. T. White, and A. A. Protter. 1993. CD36 is a receptor for oxidized low density lipoprotein. *J. Biol. Chem.* **268:** 11811–11816.
78. Febbraio, M., E. A. Podrez, J. D. Smith, D. P. Hajjar, S. L. Hazen, H. F. Hoff, K. Sharma, and R. L. Silverstein. 2000. Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. *J. Clin. Invest.* **105:** 1049–1056.
79. Febbraio, M., E. Guy, and R. L. Silverstein. 2004. Stem cell transplantation reveals that absence of macrophage CD36 is protective against atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **24:** 2333–2338.
80. Feng, J. W., J. H. Han, S. F. A. Pearce, R. L. Silverstein, A. M. Gotto, D. P. Hajjar, and A. C. Nicholson. 2000. Induction of CD36 expression by oxidized LDL and IL-4 by a common signaling pathway dependent on protein kinase C and PPAR-gamma. *J. Lipid Res.* **41:** 688–696.
81. Yesner, L. M., H. Y. Huh, S. F. Pearce, and R. L. Silverstein. 1996. Regulation of monocyte CD36 and thrombospondin-1 expression by soluble mediators. *Arterioscler. Thromb. Vasc. Biol.* **16:** 1019–1025.
82. Panousis, C. G., and S. H. Zuckerman. 2000. Regulation of cholesterol distribution in macrophage-derived foam cells by interferon-gamma. *J. Lipid Res.* **41:** 75–83.
83. Nakagawa, T., S. Nozaki, M. Nishida, J. M. Yakub, Y. Tomiyama, A. Nakata, K. Matsumoto, T. Funahashi, K. Kameda-Takemura, Y. Kurata, et al. 1998. Oxidized LDL increases and interferon-gamma decreases expression of CD36 in human monocyte-derived macrophages. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1350–1357.
84. Han, J. H., D. P. Hajjar, J. M. Tauras, J. W. Feng, A. M. Gotto, and A. C. Nicholson. 2000. Transforming growth factor-beta 1 (TGF-beta 1) and TGF-beta 2 decrease expression of CD36, the type B scavenger receptor, through mitogen-activated protein kinase phosphorylation of peroxisome proliferator-activated receptor-gamma. *J. Biol. Chem.* **275:** 1241–1246.
85. Zuckerman, S. H., C. Panousis, and G. Evans. 2001. TGF-beta reduced binding of high-density lipoproteins in murine macrophages and macrophage-derived foam cells. *Atherosclerosis.* **155:** 79–85.
86. Sawamura, T., N. Kume, T. Aoyama, H. Moriwaki, H. Hoshikawa, Y. Aiba, T. Tanaka, S. Miwa, Y. Katsura, T. Kita, et al. 1997. An endothelial receptor for oxidized low-density lipoprotein. *Nature.* **386:** 73–77.
87. Yoshida, H., N. Kondratenko, S. Green, D. Steinberg, and O. Quehenberger. 1998. Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor. *Biochem. J.* **334:** 9–13.
88. Moriwaki, H., N. Kume, H. Kataoka, T. Murase, E. Nishi, T. Sawamura, T. Masaki, and T. Kita. 1998. Expression of lectin-like oxidized low density lipoprotein receptor-1 in human and murine macrophages: upregulated expression by TNF-alpha. *FEBS Lett.* **440:** 29–32.
89. Kume, N., H. Moriwaki, H. Kataoka, M. Minami, T. Murase, T. Sawamura, T. Masaki, and T. Kita. 2000. Inducible expression of LOX-1, a novel receptor for oxidized LDL, in macrophages and vascular smooth muscle cells. *Ann. N. Y. Acad. Sci.* **902:** 323–327.
90. Chiba, Y., T. Ogita, K. Ando, and T. Fujita. 2001. PPARgamma ligands inhibit TNF-alpha-induced LOX-1 expression in cultured endothelial cells. *Biochem. Biophys. Res. Commun.* **286:** 541–546.
91. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
92. Acton, S., A. Rigotti, K. T. Landschulz, S. Z. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* **271:** 518–520.
93. van Eck, M., I. S. Bos, R. B. Hildebrand, B. T. van Rij, and T. J. van Berkel. 2004. Dual role for scavenger receptor class B, type I on bone marrow-derived cells in atherosclerotic lesion development. *Am. J. Pathol.* **165:** 785–794.
94. Zhang, W. W., P. G. Yancey, Y. R. Su, V. R. Babaev, Y. M. Zhang, S. Fazio, and M. F. Linton. 2003. Inactivation of macrophage scavenger receptor class B type I promotes atherosclerotic lesion development in apolipoprotein E-deficient mice. *Circulation.* **108:** 2258–2263.
95. Hirano, K., S. Yamashita, Y. Nakagawa, T. Ohya, F. Matsuura, K. Tsukamoto, Y. Okamoto, A. Matsuyama, K. Matsumoto, J. Miyagawa, et al. 1999. Expression of human scavenger receptor class B type I in cultured human monocyte-derived macrophages and atherosclerotic lesions. *Circ. Res.* **85:** 108–116.
96. Buechler, C., M. Ritter, C. D. Quoc, A. Agildere, and G. Schmitz. 1999. Lipopolysaccharide inhibits the expression of the scavenger receptor Cla-1 in human monocytes and macrophages. *Biochem. Biophys. Res. Commun.* **262:** 251–254.
97. Feng, B., D. J. Zhang, G. Kuriakose, C. M. Devlin, M. Kockx, and I. Tabas. 2003. Niemann-Pick C heterozygosity confers resistance to lesional necrosis and macrophage apoptosis in murine atherosclerosis. *Proc. Natl. Acad. Sci. USA.* **100:** 10423–10428.
98. Tabas, I. 2002. Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. *J. Clin. Invest.* **110:** 905–911.
99. Reiss, A. B., C. A. Patel, M. M. Rahman, E. S. Chan, K. Hasneen, M. C. Montesinos, J. D. Trachman, and B. N. Cronstein. 2004. Interferon-gamma impedes reverse cholesterol transport and promotes foam cell transformation in THP-1 human monocytes/macrophages. *Med. Sci. Monit.* **10:** BR420–BR425.
100. Yang, J. B., Z. J. Duan, W. Yao, O. Lee, L. Yang, X. Y. Yang, X. Sun, C. C. Chang, T. Y. Chang, and B. L. Li. 2001. Synergistic transcriptional activation of human acyl-coenzyme A:cholesterol acyltransterase-1 gene by interferon-gamma and all-trans-retinoic acid THP-1 cells. *J. Biol. Chem.* **276:** 20989–20998.
101. Hori, M., A. Miyazaki, H. Tamagawa, M. Satoh, K. Furukawa, H. Hakamata, Y. Sasaki, and S. Horiuchi. 2004. Up-regulation of acyl-coenzyme A:cholesterol acyltransferase-1 by transforming growth factor-beta1 during differentiation of human monocytes into macrophages. *Biochem. Biophys. Res. Commun.* **320:** 501–505.
102. Inaba, T., H. Shimano, T. Gotoda, K. Harada, M. Shimada, M. Kawamura, Y. Yazaki, and N. Yamada. 1993. Macrophage colony-stimulating factor regulates both activities of neutral and acidic cholesteryl ester hydrolases in human monocyte-derived macrophages. *J. Clin. Invest.* **92:** 750–757.
103. Björkhem, I., O. Andersson, U. Diczfalusy, B. Sevastik, R. J. Xiu, C. G. Duan, and E. Lund. 1994. Atherosclerosis and sterol 27-hydroxylase: evidence for a role of this enzyme in elimination of cholesterol from human macrophages. *Proc. Natl. Acad. Sci. USA.* **91:** 8592–8596.
104. Reiss, A. B., N. W. Awadallah, S. Malhotra, M. C. Montesinos, E. S. L. Chan, N. B. Javitt, and B. N. Cronstein. 2001. Immune complexes and IFN-gamma decrease cholesterol 27-hydroxylase in human arterial endothelium and macrophages. *J. Lipid Res.* **42:** 1913–1922.
105. Reiss, A. B., M. M. Rahman, E. S. Chan, M. C. Montesinos, N. W. Awadallah, and B. N. Cronstein. 2004. Adenosine A2A receptor occupancy stimulates expression of proteins involved in reverse cholesterol transport and inhibits foam cell formation in macrophages. *J. Leukoc. Biol.* **76:** 727–734.

106. Bellosta, S., R. W. Mahley, D. A. Sanan, J. Murata, D. L. Newland, J. M. Taylor, and R. E. Pitas. 1995. Macrophage-specific expression of human apolipoprotein E reduces atherosclerosis in hypercholesterolemic apolipoprotein E-null mice. *J. Clin. Invest.* **96:** 2170–2179.
107. Lin, C. Y., H. Duan, and T. Mazzone. 1999. Apolipoprotein E-dependent cholesterol efflux from macrophages: kinetic study and divergent mechanisms for endogenous versus exogenous apolipoprotein E. *J. Lipid Res.* **40:** 1618–1627.
108. Huang, Z. H., and T. Mazzone. 2002. ApoE-dependent sterol efflux from macrophages is modulated by scavenger receptor class B type I expression. *J. Lipid Res.* **43:** 375–382.
109. Zuckerman, S. H., G. F. Evans, and L. O'Neal. 1992. Cytokine regulation of macrophage apo-E secretion-opposing effects of GM-CSF and TGF-beta. *Atherosclerosis.* **96:** 203–214.
110. Tall, A. R. 2003. ATVB in focus—role of ABCA1 in cellular cholesterol efflux and reverse cholesterol transport. *Arterioscler. Thromb. Vasc. Biol.* **23:** 710–711.
111. Panousis, C. G., and S. H. Zuckerman. 2000. Interferon-gamma induces downregulation of Tangier disease gene (ATP-binding-cassette transporter 1) in macrophage-derived foam cells. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1565–1571.
112. Wang, X. Q., C. G. Panousis, M. L. Alfaro, G. F. Evans, and S. H. Zuckerman. 2002. Interferon-gamma-mediated downregulation of cholesterol efflux and ABC1 expression is by the Stat1 pathway. *Arterioscler. Thromb. Vasc. Biol.* **22:** E5–E9.
113. Panousis, C. G., G. Evans, and S. H. Zuckerman. 2001. TGF-beta increases cholesterol efflux and ABC-1 expression in macrophage-derived foam cells: opposing the effects of IFN-gamma. *J. Lipid Res.* **42:** 856–863.
114. Luttun, A., E. Lutgens, A. Manderveld, K. Maris, D. Collen, P. Carmeliet, and L. Moons. 2004. Loss of matrix metalloproteinase-9 or matrix metalloproteinase-12 protects apolipoprotein E-deficient mice against atherosclerotic media destruction but differentially affects plaque growth. *Circulation.* **109:** 1408–1414.
115. Corcoran, M. L., W. G. Stetler-Stevenson, P. D. Brown, and L. M. Wahl. 1992. Interleukin 4 inhibition of prostaglandin E2 synthesis blocks interstitial collagenase and 92-kDa type IV collagenase/gelatinase production by human monocytes. *J. Biol. Chem.* **267:** 515–519.
116. Yoo, H. G., B. A. Shin, J. S. Park, K. H. Lee, K. O. Chay, S. Y. Yang, B. W. Ahn, and Y. D. Jung. 2002. IL-1 beta induces MMP-9 via reactive oxygen species and NF-kappa B in murine macrophage RAW 264.7 cells. *Biochem. Biophys. Res. Commun.* **298:** 251–256.
117. Lacraz, S., L. P. Nicod, R. Chicheportiche, H. G. Welgus, and J. M. Dayer. 1995. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. *J. Clin. Invest.* **96:** 2304–2310.
118. Kumada, M., S. Kihara, N. Ouchi, H. Kobayashi, Y. Okamoto, K. Ohashi, K. Maeda, H. Nagaretani, K. Kishida, N. Maeda, et al. 2004. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation.* **109:** 2046–2049.
119. Ogawa, K., F. Chen, C. Kuang, and Y. Chen. 2004. Suppression of matrix metalloproteinase-9 transcription by transforming growth factor-beta is mediated by a nuclear factor-kappaB site. *Biochem. J.* **381:** 413–422.
120. Zhang, Y., K. McCluskey, K. Fujii, and L. M. Wahl. 1998. Differential regulation of monocyte matrix metalloproteinase and TIMP-1 production by TNF-alpha, granulocyte-macrophage CSF, and IL-1 beta through prostaglandin-dependent and -independent mechanisms. *J. Immunol.* **161:** 3071–3076.
121. Castrillo, A., S. B. Joseph, C. Marathe, D. J. Mangelsdorf, and P. Tontonoz. 2003. Liver X receptor-dependent repression of matrix metalloproteinase-9 expression in macrophages. *J. Biol. Chem.* **278:** 10443–10449.
122. Xiong, W., Y. Zhao, A. Prall, T. C. Greiner, and B. T. Baxter. 2004. Key roles of CD4(+) T cells and IFN-gamma in the development of abdominal aortic aneurysms in a murine model. *J. Immunol.* **172:** 2607–2612.
123. Shapiro, S. D., E. J. Campbell, D. K. Kobayashi, and H. G. Welgus. 1990. Immune modulation of metalloproteinase production in human macrophages. Selective pretranslational suppression of interstitial collagenase and stromelysin biosynthesis by interferon-gamma. *J. Clin. Invest.* **86:** 1204–1210.
124. Xie, B., Z. Dong, and I. J. Fidler. 1994. Regulatory mechanisms for the expression of type IV collagenases/gelatinases in murine macrophages. *J. Immunol.* **152:** 3637–3644.
125. Daugherty, A., and D. L. Rateri. 2002. T lymphocytes in atherosclerosis—the yin-yang of Th1 and Th2 influence on lesion formation. *Circ. Res.* **90:** 1039–1040.
126. Corti, R., R. Hutter, J. J. Badimon, and V. Fuster. 2004. Evolving concepts in the triad of atherosclerosis, inflammation and thrombosis. *J. Thromb. Thrombolysis.* **17:** 35–44.
127. Wilcox, J. N., K. M. Smith, S. M. Schwartz, and D. Gordon. 1989. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc. Natl. Acad. Sci. USA.* **86:** 2839–2843.
128. Toschi, V., R. Gallo, M. Lettino, J. T. Fallon, S. D. Gertz, A. Fernandez Ortiz, J. H. Chesebro, L. Badimon, Y. Nemerson, V. Fuster, et al. 1997. Tissue factor modulates the thrombogenicity of human atherosclerotic plaques. *Circulation.* **95:** 594–599.
129. Del Prete, G., M. De Carli, R. M. Lammel, M. M. D'Elios, K. C. Daniel, B. Giusti, R. Abbate, and S. Romagnani. 1995. Th1 and Th2 T-helper cells exert opposite regulatory effects on procoagulant activity and tissue factor production by human monocytes. *Blood.* **86:** 250–257.
130. Osnes, L. T., A. B. Westvik, G. B. Joo, C. Okkenhaug, and P. Kierulf. 1996. Inhibition of IL-1 induced tissue factor (TF) synthesis and procoagulant activity (PCA) in purified human monocytes by IL-4, IL-10 and IL-13. *Cytokine.* **8:** 822–827.
131. Scheibenbogen, C., H. Moser, S. Krause, and R. Andreesen. 1992. Interferon-gamma-induced expression of tissue factor activity during human monocyte to macrophage maturation. *Haemostasis.* **22:** 173–178.
132. Schwager, I., and T. W. Jungi. 1994. Effect of human recombinant cytokines on the induction of macrophage procoagulant activity. *Blood.* **83:** 152–160.
133. Whitman, S. C., P. Ravisankar, and A. Daugherty. 2002. IFN-gamma deficiency exerts gender-specific effects on atherogenesis in apolipoprotein $E^{-/-}$ mice. *J. Interferon Cytokine Res.* **22:** 661–670.
134. Gupta, S., A. M. Pablo, X. C. Jiang, N. Wang, A. R. Tall, and C. Schindler. 1997. IFN-gamma potentiates atherosclerosis in apoE knock-out mice. *J. Clin. Invest.* **99:** 2752–2761.
135. Buono, C., C. E. Come, G. Stavrakis, G. F. Maguire, P. W. Connelly, and A. H. Lichtman. 2003. Influence of interferon-gamma on the extent and phenotype of diet-induced atherosclerosis in the LDLR-deficient mouse. *Arterioscler. Thromb. Vasc. Biol.* **23:** 454–460.
136. Lee, T. S., H. C. Yen, C. C. Pan, and L. Y. Chau. 1999. The role of interleukin 12 in the development of atherosclerosis in apoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **19:** 734–742.
137. Whitman, S. C., P. Ravisankar, H. Elam, and A. Daugherty. 2000. Exogenous interferon-gamma enhances atherosclerosis in apolipoprotein $E^{-/-}$ mice. *Am. J. Pathol.* **157:** 1819–1824.
138. Whitman, S. C., P. Ravisankar, and A. Daugherty. 2002. Interleukin-18 enhances atherosclerosis in apolipoprotein $E^{-/-}$ mice through release of interferon-gamma. *Circ. Res.* **90:** E34–E38.
139. Niwa, T., H. Wada, H. Ohashi, N. Iwamoto, H. Ohta, H. Kirii, H. Fujii, K. Saito, and M. Seishima. 2004. Interferon-gamma produced by bone marrow-derived cells attenuates atherosclerotic lesion formation in LDLR-deficient mice. *J. Atheroscler. Thromb.* **11:** 79–87.
140. Linton, M. F., J. B. Atkinson, and S. Fazio. 1995. Prevention of atherosclerosis in apolipoprotein E-deficient mice by bone marrow transplantation. *Science.* **267:** 1034–1037.
141. Boisvert, W. A., J. Spangenberg, and L. K. Curtiss. 1995. Treatment of severe hypercholesterolemia in apolipoprotein E-deficient mice by bone marrow transplantation. *J. Clin. Invest.* **96:** 1118–1124.
142. Boisvert, W. A., R. Santiago, L. K. Curtiss, and R. A. Terkeltaub. 1998. A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J. Clin. Invest.* **101:** 353–363.
143. Ishibashi, M., K. Egashira, Q. Zhao, K. I. Hiasa, K. Ohtani, Y. Ihara, I. F. Charo, S. Kura, T. Tsuzuki, A. Takeshita, et al. 2004. Bone marrow-derived monocyte chemoattractant protein-1 receptor CCR2 is critical in angiotensin II-induced acceleration of atherosclerosis and aneurysm formation in hypercholesterolemic mice. *Arterioscler. Thromb. Vasc. Biol.* **24:** e174–e178.
144. Sata, M., A. Saiura, A. Kunisato, A. Tojo, S. Okada, T. Tokuhisa, H. Hirai, M. Makuuchi, Y. Hirata, and R. Nagai. 2002. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. *Nat. Med.* **8:** 403–409.

145. Rauscher, F. M., P. J. Goldschmidt Clermont, B. H. Davis, W. Tang, D. Gregg, R. Ramaswami, A. M. Pippen, B. H. Annex, C. M. Dong, and D. A. Taylor. 2003. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation.* **108:** 457–463.
146. Zhang, D., W. Tang, P. M. Yao, C. W. Yang, B. X. Xie, S. Jackowski, and I. Tabas. 2000. Macrophages deficient in CTP:phosphocholine cytidylyltransferase-alpha are viable under normal culture conditions but are highly susceptible to free cholesterol-induced death—molecular genetic evidence that the induction of phosphatidylcholine biosynthesis in free cholesterol-loaded macrophages is an adaptive response. *J. Biol. Chem.* **275:** 35368–35376.
147. Kanters, E., M. Pasparakis, M. J. J. Gijbels, M. N. Vergouwe, I. Partouns Hendriks, R. J. A. Fijneman, B. E. Clausen, I. Forster, M. M. Kockx, K. Rajewsky, et al. 2003. Inhibition of NF-kappa B activation in macrophages increases atherosclerosis in LDL receptor-deficient mice. *J. Clin. Invest.* **112:** 1176–1185.
148. Draude, G., and R. L. Lorenz. 2000. TGF-beta 1 downregulates CD36 and scavenger receptor A but upregulates LOX-1 in human macrophages. *Am. J. Physiol. Heart Circ. Physiol.* **278:** H1042–H1048.
149. Nishimura, N., M. Harada Shiba, S. Tajima, R. Sugano, T. Yamamura, Q. Z. Qiang, and A. Yamamoto. 1998. Acquisition of secretion of transforming growth factor-beta 1 leads to autonomous suppression of scavenger receptor activity in a monocyte-macrophage cell line, THP-1. *J. Biol. Chem.* **273:** 1562–1567.
150. Bottalico, L. A., R. E. Wager, L. B. Agellon, R. K. Assoian, and I. Tabas. 1991. Transforming growth factor-beta1 inhibits scavenger receptor activity in THP-1 human macrophages. *J. Biol. Chem.* **266:** 22866–22871.
151. Liao, H. S., A. Matsumoto, H. Itakura, T. Doi, M. Honda, T. Kodama, and Y. J. Geng. 1999. Transcriptional inhibition by interleukin-6 of the class A macrophage scavenger receptor in macrophages derived from human peripheral monocytes and the THP-1 monocytic cell line. *Arterioscler. Thromb. Vasc. Biol.* **19:** 1872–1880.
152. de Villiers, W. J. S., I. P. Fraser, D. A. Hughes, A. G. Doyle, and S. Gordon. 1994. Macrophage-colony-stimulating factor selectively enhances macrophage scavenger receptor expression and function. *J. Exp. Med.* **180:** 705–709.
153. de Villiers, W. J. S., I. P. Fraser, and S. Gordon. 1994. Cytokine and growth factor regulation of macrophage scavenger receptor expression and function. *Immunol. Lett.* **43:** 73–79.
154. Minami, M., N. Kume, H. Kataoka, M. Morimoto, K. Hayashida, T. Sawamura, T. Masaki, and T. Kita. 2000. Transforming growth factor-beta(1) increases the expression of lectin-like oxidized low-density lipoprotein receptor-1. *Biochem. Biophys. Res. Commun.* **272:** 357–361.
155. Higuchi, S., A. Tanimoto, N. Arima, H. Xu, Y. Murata, T. Hamada, K. Makishima, and Y. Sasaguri. 2001. Effects of histamine and interleukin-4 synthesized in arterial intima on phagocytosis by monocytes/macrophages in relation to atherosclerosis. *FEBS Lett.* **505:** 217–222.
156. Wuttge, D. M., X. Zhou, Y. Sheikine, D. Wagsater, V. Stemme, U. Hedin, S. Stemme, G. K. Hansson, and A. Sirsjo. 2004. CXCL16/SR-PSOX is an interferon-gamma-regulated chemokine and scavenger receptor expressed in atherosclerotic lesions. *Arterioscler. Thromb. Vasc. Biol.* **24:** 750–755.
157. Brand, K., N. Mackman, and L. K. Curtiss. 1993. Interferon-gamma inhibits macrophage apolipoprotein-E production by posttranslational mechanisms. *J. Clin. Invest.* **91:** 2031–2039.
158. Duan, H. W., Z. G. Li, and T. Mazzone. 1995. Tumor necrosis factor-alpha modulates monocyte/macrophage apoprotein E gene expression. *J. Clin. Invest.* **96:** 915–922.