Signal Transducer and Activator of Transcription 1 Is Required for Optimal Foam Cell Formation and Atherosclerotic Lesion Development

Sudesh Agrawal, PhD; Maria Febbraio, PhD; Eugene Podrez, MD, PhD; Martha K. Cathcart, PhD; George R. Stark, PhD; Guy M. Chisolm, PhD

Background—Signal transducer and activator of transcription 1 (Stat1) potently regulates gene expression after stimulation by certain cytokines involved in tumorigenesis and host defenses. The present study investigated a novel role for Stat1 in foam cell formation and atherosclerosis.

Methods and Results—Inhibition of Stat1 activity by a Stat1-specific DNA “decoy” oligomer transfected into differentiated human THP-1 cells, and deficiency of stat1 in mouse macrophages significantly inhibited foam cell formation assessed by lipid staining and cholesteryl ester accumulation compared with control cells. The mechanism of Stat1 regulation of foam cell formation was uniquely dependent on the scavenger receptor CD36. Blunted Stat1 activity and stat1 deficiency significantly decreased expression of CD36 but not of scavenger receptor-A compared with controls, as assessed by immunoblotting and flow cytometry. Deficiency of CD36 but not scavenger receptor-A in mouse macrophages removed any dependency of foam cell formation on Stat1. In an intraperitoneal model of foam cell formation in which foam cells form in vivo independently of the model ligands used in vitro, stat1 deficiency significantly inhibited foam cell formation and CD36 expression. Transplantation of bone marrow from apolipoprotein e⁻/⁻ × stat1⁻/⁻ mice into lethally irradiated, atherosclerosis-susceptible apolipoprotein e⁻/⁻ recipients significantly reduced both en face aortic lesion coverage and aortic root lesions compared with recipients of bone marrow from genetically matched apolipoprotein e⁻/⁻ mice.

Conclusions—Stat1 regulates CD36 expression and foam cell formation in macrophages in vitro; the Stat1 regulation of foam cell formation was uniquely dependent on the scavenger receptor CD36. Blunted Stat1 activity and atherosclerosis-susceptible bone marrow transplantation model. (Circulation. 2007;115:2939-2947.)

Key Words: atherosclerosis ■ CD36 antigens ■ foam cells ■ lipoproteins ■ macrophage ■ signal transduction ■ STAT1 transcription factor

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In early fatty streak lesions of human and mouse atherosclerosis, lipoprotein accumulation and oxidation occur. This is followed by activation of the endothelium, recruitment of circulating monocytes and their adhesion to the endothelium, diapedesis into the intima, and differentiation to macrophages.7,8 Macrophage uptake of oxidized lipoproteins via scavenger receptors such as CD36 and scavenger receptor-A (SR-A)9,10 is hypothesized to be an early event in atherosclerotic lesion formation,11 which leads to the formation of lipid-engorged “foam cells.” The studies herein tested the hypothesis that Stat1 is proatherogenic. The role of Stat1 was studied in vitro and in vivo foam cell formation model systems and an

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Methods

THP-1 Cell Differentiation and Foam Cell Formation
THP-1 cells obtained from American Type Culture Collection (ATCC, Manassas, Va.) were differentiated by stimulation with 15 nM phorbol 12-myristate 13-acetate (PMA) for 2 hours and then washed with PBS and cultured in RPMI-1640 with 10% fetal bovine serum for multiple days. Foam cell formation was induced by incubation of differentiated THP-1 cells (grown on glass coverslips) for 48 hours with cupric ion-oxidized low-density lipoprotein (LDL) (Cu-oxLDL; 50 μg/mL) or other forms of modified LDL. Cells were stained with Oil Red O as described.12 Photographs of foam cells were taken with a phase-contrast microscope, and at least 10 microscopic fields were counted from 4 different slides for the same treatment for quantification of foam cells.

Flow Cytometry
Washed THP-1 cells were incubated with phycoerythrin-conjugated anti-human CD11b or fluorescein isothiocyanate–conjugated anti-human CD36 antibodies for 60 minutes on ice in the dark. Mouse bone marrow macrophages (BMMs) were incubated first with a mouse anti-mouse CD36 polyclonal antibody and second with a phycoerythrin-conjugated anti-mouse IgA, and mouse IgA was used as isotype control (all antibodies were from BD Biosciences, San Jose, Calif). At least 25 000 cells were analyzed on a Becton-Dickinson FACScan with CellQuest software.

Transfection of a Stat1 Decoy and Missense Oligodeoxyribonucleotides Into THP-1 and Mouse Primary Macrophages
A double-stranded DNA oligomer, used successfully as a DNA “decoy” in previous studies, was used to specifically inhibit Stat1 activity.13,14 The phosphorothioated oligodeoxyribonucleotides, the decoy and missense control oligomers, were purchased from Sigma-Genosys Biotechnologies, Inc (Woodlands, Tex).

Gel Shift Assay
For detection of Stat1 DNA binding to protein, a gel shift assay was performed as described.14,15 The probe used for Stat1 DNA binding was the consensus binding site for Stat1, double-stranded DNA oligomer, was purchased from Sigma. The reaction contained 3 μL of genomic DNA and 0.5 μL of the pooled primers at 50 pmol/μL each. The Expand High Fidelity PCR system kit (Roche Diagnostics) was used. The reaction mixture was preheated to 94°C for 3 minutes, then run for 31 cycles at 94°C for 1 minute, 60°C for 1 minute, 72°C for 3 minutes, and then again at 72°C for 3 minutes at the end of the reaction. The products were run on a 2% agarose gel to distinguish the 285-bp wild-type apo e allele product from the recipient mice posttransplantation (see Animal Genotyping and PCR below). PCR analysis performed as previously described22,23 revealed 75% to 100% engraftment of apo e or apo e/×stat1−/− bone marrow into lethally irradiated apo e recipient mice (2 mice that received stat1−/− bone marrow did not show significant engraftment of the stat1−/− allele; data from these 2 mice were excluded).

Animal Genotyping and PCR
Genotyping was performed on ear clip–derived DNA. For apo e genotyping, the PCR protocol described on the Jackson Laboratory Web site was used. For stat1 genotype PCR analysis, 2 different reactions were used: one that used the stat1 sense primer 5′-CTTCTAACCCTTCTGACAC-3′ and antisense primer 5′-CCTTTGCACTTTCTGAGACC-3′ to detect the wild-type allele, and one that used the stat1 sense primer 5′-CTTCCAGATCTCTGCTTAGAC-3′ and antisense primer 5′-GCGCCTCCCGTATTGCA-GCGCAGTCCGC-3′. The reaction contained 3 μL of genomic DNA and 0.5 μL of the pooled primers at 50 pmol/μL each. The Expand High Fidelity PCR system kit (Roche Diagnostics) was used. The reaction mixture was preheated to 94°C for 3 minutes, then run for 31 cycles at 94°C for 1 minute, 60°C for 1 minute, 72°C for 3 minutes, and then again at 72°C for 3 minutes at the end of the reaction. The products were run on a 2% agarose gel to distinguish the 285-bp wild-type apo e allele product from the 570-bp knockout allele product.

Atherosclerosis Lesion Measurements
Atherosclerotic lesions were quantified in bone marrow transplanted mice by 2 independent and blinded assessments: en face aortic coverage measured by computer-assisted planimetry, as described by...
Results

Blunting Stat1 Binding to DNA Inhibited Foam Cell Formation in Differentiated Human Monocytoid THP-1 Cells

To determine if Stat1 can regulate foam cell formation, a frequently studied in vitro foam cell model system was used: the human monocyte-like leukemic cell line THP-1. Stat1 had been shown to be involved early in cell differentiation; therefore, the effects of Stat1 on foam cell formation were only tested after differentiation. The time course and degree of Stat1 activation were determined after differentiation was initiated with PMA. Differentiation was monitored by CD11b/MAC-1 expression with use of fluorescence-activated cell-sorting analysis. CD11b reached a maximum and a plateau at 3 days (Figure 1A). Stat1 activation was assessed by immunoblot analysis with use of antibodies specific for Stat1 phosphorylation at serine-727 and tyrosine-701 and by measurement of binding of a radiolabeled DNA Stat1 consensus binding element to proteins in nuclear extracts. In both assays, adherent THP-1 cells expressed a relatively low but detectable basal Stat1 activity. This increased markedly in response to treatment with PMA; however, after 2 days Stat1 activation returned to basal levels (data not shown). Thus, to test the effect of Stat1 on foam cell formation independently of the effect of Stat1 on differentiation, experiments were conducted after 3 days.

Foam cell formation was induced postdifferentiation by exposure of THP-1-derived macrophages to Cu-oxLDL for 2 days. A double-stranded DNA decoy oligomer was transfected into differentiated THP-1 cells to block Stat1 binding to its target genes. The effectiveness of this decoy was assessed by measurement of the binding of a radiolabeled DNA Stat1 consensus binding element to proteins in the nuclear extract. Transfection of the Stat1 decoy reduced Stat1 binding markedly (80% to 85%) compared with differentiated THP-1 cells that were untreated, treated with Superfectin alone, or transfected with missense double-stranded DNA (Figure 1B). Foam cell formation, as identified by Oil Red O staining, was readily apparent in cells treated with Cu-oxLDL alone, Cu-oxLDL plus Superfectin, and Cu-oxLDL plus missense oligomer. Foam cell formation was reduced in cells treated with Cu-oxLDL plus the Stat1 decoy compared with the above-mentioned control cells (Figure 1C). Cholesteryl ester accumulation, also used to quantify foam cell formation, was significantly decreased by ~50% in Cu-oxLDL–incubated cells treated with the Stat1 decoy compared with Cu-oxLDL–incubated control, missense-treated, or Superfectin-treated cells (Figure 1D).

Stat1 Deficiency Inhibited Foam Cell Formation in Mouse Bone Marrow Macrophages

In an independent approach, BMMs from wild-type and stat1−/− mice (SV129 genetic background) were obtained by harvesting of marrow cells and inducing differentiation. After 5 days, macrophages were treated with Cu-oxLDL for 48 hours to induce foam cell formation. Lipid accumulation, assessed with Oil Red O (see Methods), was 60% to 70% less in BMMs from stat1−/− mice than in those from wild-type mice (Figure 2A). Cholesteryl ester accumulation after Cu-
oxLDL treatment was also attenuated in BMMs from stat1/−/− mice compared with those from wild-type mice (5.8±0.5 μg/mg versus 9.8±1.7 μg/mg of protein; P<0.008) (Figure 2B). Similar results were obtained with use of MPMs from stat1/−/− and wild-type mice (data not shown).

**CD36 Expression Was Inhibited in THP-1 Cells After Stat1 Activity Was Blunted**

Macrophages recognize and selectively ingest modified lipoproteins through multiple cell surface scavenger receptors to become foam cells. Deficiencies of either of 2 such receptors, SR-A and CD36, have been reported to reduce lesions in atherosclerosis-susceptible mouse models.29,30 We hypothesized that the mechanism of decreased foam cell formation after interference with Stat1 could be a result of Stat1 regulation of 1 or both receptors. Immunostaining for CD36 by flow cytometry revealed marginally reduced basal and significantly reduced Cu-oxLDL–induced CD36 expression on Stat1-decoy–treated and differentiated THP-1 cells compared with sham-treated control cells (Figure 3A).

**CD36 Expression Was Inhibited in Bone Marrow Macrophages From stat1/−/− Mice Compared With Those From Wild-Type Mice**

BMMs from stat1/−/− mice exposed to Cu-oxLDL showed significantly reduced basal expression of CD36 compared with those from wild-type mice by flow cytometry (Figure 3B) and Western blot analysis (Figure 3C). Cu-oxLDL enhanced CD36 protein expression in a time-dependent manner in BMMs from stat1/−/− mice and wild-type mice, but the level of induction was significantly less in stat1/−/− macrophages. Expression of SR-A was not affected by the absence of Stat1, as analyzed by immunoblotting and flow cytometry (data not shown). These data showed that at least a correlation existed between reduced Stat1 activity or Stat1 deficiency, reduced CD36 expression, and reduced foam cell formation.

To test whether decreased CD36 expression after interference with Stat1 resulted in reduced CD36 function (ie, decreased binding and cell association of CD36 ligands), particular forms of modified LDL that have been shown to be recognized by distinct scavenger receptors were used.12 It has been shown that LDL does not bind SR-A or CD36; Ac-LDL is a preferential ligand for SR-A; Cu-oxLDL is a ligand for both SR-A and CD36; and NO2-oxLDL is a preferential ligand for CD36.12 Thioglycollate-elicited MPMs from stat1/−/− or wild-type mice were incubated with 75 μg/mL of the 125I-labeled ligands, LDL, Ac-LDL, or NO2-oxLDL, or with 50 μg/mL 125I-labeled Cu-oxLDL. Ligand binding (incubation at 4°C) and cell association (ie, binding and uptake

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**Figure 2.** Foam cell formation was blunted in macrophages from stat1-deficient mice compared with wild-type mice. A, Staining with Oil Red O allowed visualization of cytoplasmic lipid accumulation. Scale bar=10 μm. B, Foam cell formation was quantified by cholesteryl ester:protein ratio. Data are means±SD from triplicate samples.

**Figure 3.** Macrophage CD36 expression was blunted by Stat1 inhibition or stat1/−/− deficiency. A, Differentiated THP-1 cells were treated with Stat1 decoy or missense control and labeled with anti-CD36 antibody. CD36 was analyzed by flow cytometry. Data are means±SD of triplicate samples and expressed as percentages of CD36 positive cells. B, Peritoneal macrophages were collected from wild-type and stat1/−/− mice, incubated with fluorescein isothiocyanate–labeled anti-CD36 antibody, and CD36 expression was analyzed by flow cytometry. C, Peritoneal macrophages from stat1/−/− and wild-type mice were treated with Cu-oxLDL for the times indicated. Immunoblot analysis was performed with anti-CD36 polyclonal antibody (developed and characterized by M.F.; unpublished observations, 2006). The blots were stripped and reprobed with anti-GAPDH polyclonal antibody to assess loading uniformity.
after incubation at 37°C) were quantified as described. Binding of 125I-labeled Ac-LDL did not differ between wild-type and stat1−/− macrophages. In contrast, binding of 125I-labeled NO2-oxLDL and binding of 125I-labeled Cu-oxLDL were both significantly decreased in macrophages from stat1−/− mice compared with macrophages from wild-type mice (Figure 4A). Analogous results were obtained with BMMs from stat1−/− and wild-type mice (data not shown). The relationship between Stat1 regulation of CD36 expression, CD36 ligand recognition, and foam cell formation was probed further by treatment of BMMs from stat1−/− and wild-type mice with 50 μg/mL unlabeled Cu-oxLDL, NO2-oxLDL, or Ac-LDL for 24 hours. Oil Red O staining revealed that the number of lipid-engorged foam cells was similar in Ac-LDL treated wild-type and stat1−/− macrophages. In contrast, a 40% to 45% (P<0.025) decrease was observed in the number of foam cells in stat1−/− macrophages treated with Cu-oxLDL and a 60% to 65% (P<0.003) decrease in stat1−/− macrophages treated with NO2-oxLDL compared with macrophages from wild-type mice (Figure 4B). These data are consistent with the interpretation that Stat1 regulates the expression of CD36 but not SR-A, and that the altered expression has functional impact through binding and uptake of CD36-recognized lipoproteins.

**Decreased Foam Cell Formation in Macrophages From stat1−/− Mice Was Caused by Inhibition of CD36, but not SR-A; Deficiency of CD36 Abolished Dependency of Foam Cell Formation on Stat1**

To determine whether regulation of CD36 by Stat1 was required for the Stat1 effect on foam cell formation, and to determine whether Stat1-regulated proteins other than CD36 markedly influenced foam cell formation, thioglycollate-elicited MPMs were isolated from background-matched sr-a−/−, cd36−/−, sr-a−/−×cd36−/−, and wild-type mice, and replicate cultures were treated with the Stat1 DNA oligomer “decoy.” Foam cell formation was unchanged by decoy treatment of macrophages from cd36−/− or cd36−/−×sr-a−/− mice. Specificity of the decoy was evaluated as shown in Figure 1B. Results shown are expressed as cholesteryl ester:protein ratios and are means±SD of data combined from 2 experiments.
formation was significantly inhibited by the Stat1 decoy in MPMs from wild-type and sr-aI mice (Figure 4C). In contrast, no significant inhibition of foam cell formation was observed in Stat1 decoy-treated MPMs from cd36I−I or double-knockout mice (Figure 4C); the absence of CD36 removed the Stat1 dependency of foam cell formation. Similar results were observed in Stat1 decoy-treated BMMs from these animals (data not shown). These data demonstrated a requirement for CD36 in Stat1 regulation of foam cell formation and revealed that, in the absence of CD36, blockade of Stat1 activity did not inhibit foam cell formation by CD36-independent means.

Foam Cell Formation and CD36 Expression Were Inhibited In Vivo by Stat1 Deficiency

To determine whether foam cell formation in vivo was limited by stat1 deficiency, and whether CD36 expression was reduced in vivo by stat1 deficiency, a variation of the technique reported by Li et al was adapted (see Methods). Six days after peritoneal thioglycollate injection into either apoeI−I mice or apoeI−I×stat1I−I mice, peritoneal cells were harvested and assessed with Oil Red O staining and cholesteryl ester:protein measurement after allowing in vivo foam cell formation. Cell surface CD36 expression and plasma cholesterol were also quantified. Cholesteryl ester accumulation was significantly reduced in peritoneal cells from apoeI−I×stat1I−I mice compared with those from apoeI−I mice (1.29±0.1 µg/mg cell protein (n=12) versus 2.65±0.1 µg/mg (n=11); P<0.003) (Figure 5A), as was CD36 cell surface expression (546.3±79.2 relative units of intensity (n=12) versus 1102±164.0 (n=11); P<0.005) (Figure 5B). No significant difference existed in the total number of peritoneal cells harvested (27±1.7×106 versus 28.2±2.0×106; P<0.15) or plasma cholesterol levels (1869±239 µg/mL [n=12] versus 2194±120 µg/mL [n=11]; P<0.25) between the apoeI−I and apoeI−I×stat1I−I mice. Thus, Stat1 deficiency is linked to reduced CD36 expression on foam cells formed in vivo, analogous to the results obtained in vitro. In addition, the role of Stat1 in foam cell formation that was observed with the model of lipoproteins oxidized in vitro was recapitulated when foam cells were produced from ligands formed in vivo.

Atherosclerotic Lesion Formation Was Inhibited in apoeI−I Mice That Received Bone Marrow From apoeI−I×stat1I−I Compared With apoeI−I Mice That Received Bone Marrow From apoeI−I Mice

Eight-week-old apoeI−I mice were irradiated and received bone marrow transplants. After 4 weeks, they were fed a high-fat diet for an additional 14 week-old. apoeI−I mice that received bone marrow from apoeI−I×stat1I−I mice had significantly reduced en face aortic lesion area coverage compared with recipients of bone marrow from apoeI−I mice (1.41±0.4% (n=10) versus 4.33±0.8% (n=12), which is a 69% decrease (P<0.004) (Figure 6, A to C). Aortic root lesions were reduced from 274 400±24 360 µm2 (n=12) in apoeI−I recipients to 165 100±18 740 µm2 (n=10) in apoeI−I×stat1I−I recipients (Figure 6D through 6F), which is a 45% decrease (P<0.0026). Surprisingly, compared with apoeI−I mice that received apoeI−I bone marrow, mice that received apoeI−I×stat1I−I bone marrow were statistically significantly heavier (Figure 6H) and had significantly higher plasma cholesterol levels (Figure 6G). Immunohistochemistry with anti–phospho-Stat1 revealed that phosphorylated Stat1 was present in aortic root lesions from apoeI−I mice but not in aortic root lesions from apoeI−I×stat1I−I mice (data not shown).

Discussion

Our results reveal 2 novel findings. First, in both in vitro and in vivo macrophage cell systems, interference with Stat1-dependent pathways downregulated CD36 expression. Reduced foam cell formation in vitro by Stat1 pathway disruption depended on CD36. Second, our in vivo results showed that Stat1 deficiency reduced foam cell formation in an intraperitoneal inflammation model and reduced atherosclerosis in an atherosclerosis-susceptible bone marrow transplantation mouse model.

Our discovery that Stat1 helps regulate CD36 and foam cell formation is reinforced by the consistency of the findings in 3 distinct in vitro models (differentiated human THP-1 cells, BMMs, and MPMs), with use of different inducers of macrophage differentiation (PMA, granulocyte-macrophage colony-stimulating factor–rich L929-conditioned media, and thioglycollate recruitment and differentiation in vivo) and use of different means of Stat1 pathway interference (blockade of Stat1 binding to DNA and Stat1 deficiency). Consistent results were also obtained in an in vivo peritoneal inflammation model of foam cell formation, in which foam cell formation occurred in vivo, independent of in vitro–modified model lipoproteins.
Our results show by multiple approaches that the mechanism by which Stat1 regulates foam cell formation involves CD36 and that CD36 was a required element in the regulatory pathway. The effect of Stat1 on foam cell formation was absent in macrophages deficient in CD36, but not in macrophages deficient in SR-A. This effect of Stat1 on CD36 is likely indirect. The CD36 promoter does not contain a known consensus Stat1 binding motif. Such a binding motif is present in the SR-A promoter32; however, we did not find evidence of Stat1 regulation of SR-A expression.

The discovery of the regulation of CD36 by Stat1 has potentially far-reaching implications that are independent of atherosclerosis. CD36 plays a role in a number of important pathological and physiological processes, such as long-chain fatty acid transport, recognition and clearance of apoptotic cells, sequestration of the malarial parasite, and immune responses to infections.9,33,34 CD36 has been implicated in the pathogenesis of diabetes mellitus and the metabolic syndrome by virtue of its effect on fatty acid uptake and utilization, and is critical in the ability of tissues to meet their energy needs during stress or exercise.9,33,34 If CD36 is regulated by Stat1 in contexts other than atherosclerosis, their elucidation could lead to enhanced understanding of these physiological and pathophysiological processes and may provide alternative approaches to development of targeted drug therapy.

A well-established role for Stat1 has been demonstrated in tumorigenesis and host-defense mechanisms,35,36 but its role in atherosclerosis is a novel observation. Our findings are of particular interest in light of multiple prior observations. Stat1 is a downstream target in IFN-γ signaling, and several studies have identified a role for IFN-γ in atherosclerosis, such as in atherosclerosis-susceptible mice.4–6 Our data could be construed to suggest that Stat1 represents a step in the intracellular pathway by which IFN-γ or other cytokines worsen fatty streak formation and that CD36 might mediate this process. However, in vitro reports of the effect of IFN-γ on CD36 are inconclusive. IFN-γ has been shown to enhance37 and to decrease38 CD36 in various monocyte and macrophage-like cell systems. In addition, there are other possible explanations. Stat1 may be proatherogenic by an alternative mechanism. Stat1 activity was recently shown to contribute to 15-lipoxygenase expression,14 and 15-lipoxygenase expression is believed to be atherogenic.39,40

On the basis of our in vitro findings that CD36 mediates the reduced foam cell formation caused by interference with Stat1 pathways, it is tempting to speculate that the reduction in atherosclerosis we observed in Stat1 deficiency was also mediated by CD36. However, this would be premature. Although reduced foam cell lesions in atherosclerosis-susceptible mice deficient in either CD3630 or SR-A29 have been reported, a recent paper has questioned the role of scavenger receptors in atherosclerosis.41 In that study, in the absence of CD36 en face aortic analysis showed significant protection from atherosclerosis in female mice, but only a
trend toward reduced lesion area in males. Furthermore, the aortic root lesion data were discordant; females that lacked CD36 had larger lesions, and no significant difference was found in males.41 Thus, our data could reflect other CD36-independent and antiatherogenic influences exerted by the deficiency in stat1.

Our data highlight the idea that Stat1 inhibition could represent a target to reduce inflammation. Certain substances known to inhibit Stat1, albeit nonselectively, namely the 3-hydroxy-3-methylglutaryl coenzyme A reductase-inhibiting statins42 and epigallocatechin-3-gallate,43 have also been shown to inhibit atherosclerosis in humans and animals.44,45 Statins are known to have antiatherosclerotic effects in excess of that predicted from their effects on LDL lowering; our results invite speculation that a part of these pleiotropic effects of statins could be linked to Stat1 inhibition.

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Disclosures

None.

References


Signal transducer and activator of transcription 1 (Stat1) is a very well-studied transcription factor in the janus kinase–Stat signaling pathway. It is a downstream target of interferon-γ and other specific cytokines and regulates numerous genes involved in immune responses, tumorigenesis, and inflammation. The results reported here reveal for the first time that Stat1 regulates the conversion of macrophages to lipid-engorged “foam cells,” an early cellular event in the pathological sequence of atherosclerotic lesion development. Furthermore, mice deficient in Stat1 developed significantly less atherosclerosis than did control mice in a bone marrow transplantation model of atherosclerosis. The in vitro data presented here show, also for the first time, that Stat1 regulates the scavenger receptor CD36, a protein linked to fatty acid transport, apoptotic cell recognition, metabolic syndrome, and responses to infection. The potential significance of these findings includes the possibility that further studies of Stat1 regulation of CD36 and its influence on atherosclerosis will identify novel therapeutic targets in pathological Stat1-dependent signaling pathways. Interestingly, certain substances known to inhibit Stat1, albeit nonselectively (eg, 3-hydroxy-3-methylglutaryl coenzyme A reductase-inhibiting statins and epigallocatechin-3-gallate) have also been shown to inhibit atherosclerosis in humans and animals. Because statins are known to have antiatherosclerotic effects in excess of that predicted from their effects on lowering low-density lipoprotein, it is tempting to speculate that a part of the pleiotropic effects of statins could be through Stat1 inhibition.
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