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Plasma from systemic lupus patients compromises cholesterol homeostasis: a potential mechanism linking autoimmunity to atherosclerotic cardiovascular disease

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Abstract

Atherosclerotic cardiovascular disease (ASCVD) contributes to morbidity and mortality in systemic lupus erythematosus (SLE). Immunologic derangements may disrupt cholesterol balance in vessel wall monocytes/macrophages and endothelium. We determined whether lupus plasma impacts expression of cholesterol 27-hydroxylase, an anti-atherogenic cholesterol-degrading enzyme that promotes cellular cholesterol efflux, in THP-1 human monocytes and primary human aortic endothelial cells (HAEC). THP-1 monocytes and HAEC were incubated in medium containing SLE patient plasma or apparently healthy control human plasma (CHP). SLE plasma decreased 27-hydroxylase message in THP-1 monocytes by 47 ± 8% ($p < 0.008$) and in HAEC by 51 ± 5.5% ($n = 5$, $p < 0.001$). THP-1 macrophages were incubated in 25% lupus plasma or CHP and cholesterol-loaded (50 μg ml^{-1} acetylated low density lipoprotein). Lupus plasma more than doubled macrophage foam cell transformation (74 ± 3% vs.35 § 3% for CHP, $n = 3$, $p < 0.001$). Impaired cholesterol homeostasis in SLE provides further evidence of immune involvement in atherogenesis. Strategies to inhibit or reverse arterial cholesterol accumulation may benefit SLE patients.

Keywords

Lupus erythematosus; Systemic; Atherosclerosis; Cholesterol; Macrophage scavenger receptor; Foam cells

Introduction

Premature atherosclerotic cardiovascular disease (ASCVD) is a common and devastating complication of systemic lupus erythematosus (lupus, SLE) which occurs despite the normal to low total cholesterol levels found in a majority of persons with lupus [1, 2]. Chronic active inflammation contributes to premature ASCVD in these patients, possibly by disrupting homeostatic mechanisms that orchestrate cholesterol balance in the vessel wall.

Treatment for lupus has improved, and short-term prognosis has increased from less than 50% survival at 5 years to 93% at 5 years, and 85% at 10 years [3]. However, many patients who survive early complications of this autoimmune disease experience considerable late morbidity and mortality from cardiovascular events including angina and myocardial infarction (MI) [4]. Premature ASCVD in SLE is a major public health concern and

premenopausal women with SLE were over 50 times more likely to have a myocardial infarction than were women of similar age in the Framingham Offspring Study (rate ratio = 52.43, 95% con-fidence interval 21.6–98.5) [5, 6]. Atherosclerosis is the most common type of coronary artery pathology in SLE [7, 8]. Non-atherosclerotic disease processes such as coronary vasculitis may also be important [9]. Coronary dissection and coronary artery aneurysm are rare, but may occur [10, 11]. Although mechanisms of vasculopathy in SLE are not completely understood, a number of lupus-associated factors may play a part. Potential mechanisms involved in the pathophysiology of coronary artery disease in lupus include microvascular disease, coronary aneurysms, intracoronary thrombosis, or the result of pharmacotherapy such as corticosteroids [12, 13].

The role of immunological mechanisms in atherosclerosis in these patients needs further elucidation, but inflammatory processes are known to accelerate development of atheroma [14]. Deposition of immune complexes may lead to intimal damage [15].

Antiphospholipid antibodies (APLs) have been implicated in arterial thrombosis, including premature coronary artery and cerebrovascular thrombosis [14]. A hypercoagulable state leading to coronary thrombosis may also be associated with antiphospholipid syndrome or renal involvement in lupus [16]. Anticardiolipin antibody (found in 30–40% of SLE patients) and their crossreactivity with oxidized low density lipoprotein (LDL) antibody provide a possible link between the thrombotic and atherosclerotic sequelae of SLE [17–19]. Known risk factors for ASCVD that occur with greater frequency in SLE patients than in the general population include corticosteroid-induced hypercholesterolemia and hyperglycemia as well as hypertension associated with renal disease. However, even in studies controlling for steroid therapy and renal disease, the association between SLE and accelerated atherosclerosis, especially in premenopausal women who are generally at low risk, is inordinately high [20]. The diagnosis of SLE is itself a strong risk factor for ASCVD. Many lupus patients have normal or low total cholesterol levels and although vasculitis rather than lipid abnormalities accounts for some of the thrombotic events in lupus patients, a majority of afflicted patients develop lesions histologically indistinguishable from ordinary atherosclerotic plaques [21, 22].

We previously reported that specific immune reactants that play a role in the pathogenesis of SLE downregulate the reverse cholesterol transport proteins cholesterol 27-hydroxylase and ATP binding cassette transporter 1 (ABCA1) in cell types relevant to atherogenesis [23, 24]. The mitochondrial cytochrome P450 cholesterol 27-hydroxylase defends cells against accumulation of excess cholesterol, making this enzyme of particular interest as a target in the management of dyslipidemia [25]. We were among the first to report expression of cholesterol 27-hydroxylase in primary human arterial endothelium, an early indication that endothelial cells participate in cholesterol metabolism in the vessel wall [26, 27]. Lipid accumulation in arteries induces vascular inflammation and atherosclerosis. The process begins with endothelial cell activation and monocyte recruitment, followed by excessive lipoprotein uptake by macrophages leading to fatty streak formation [28, 29]. Hypercholesterolemia is associated with endothelial dysfunction [30, 31]. Expression of 27-hydroxylase by endothelium and monocytes/macro-phages can reduce the lipid burden on these cells, providing a defense mechanism against atherosclerosis [32]. We report here that plasma from lupus patients has atherogenic properties. Cultured THP-1 human monocytes and arterial endothelium exposed to lupus plasma exhibit a decrease in mRNA and protein for cholesterol 27-hydroxylase while THP-1 macrophages show an increase in foam cell transformation when lipid-loaded.

Methods

Cell culture

THP-1 cells (American Type Culture Collection Rockville, MD) and human aortic endothelial cells (HAEC, Cambrex Bio Science Walkersville, MD) were grown at 37°C in a 5% CO_2 atmosphere to a density of 10^6 cells ml^{-1}. Growth medium for THP-1 cells was RPMI 1640 (GIBCO BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) from the same source, 50 units ml^{-1} penicillin, and 50 units ml^{-1} streptomycin. Growth medium for HAEC was endothelial growth medium-2 (EGM-2, Cambrex, Inc.).

Human blood samples

Subject inclusion and exclusion criteria—The research has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Human subject studies were performed under a protocol approved by the Institutional Review Boards of Winthrop University Hospital, New York University School of Medicine and the Oklahoma Medical Research Foundation. Written informed consent was obtained from all subjects.

Levels of 27-hydroxylase protein were determined in cultured THP-1 human monocytoid cells or HAEC after exposure to plasma from SLE and control female subjects.

Apparently healthy subjects: Volunteers, age 18–29, not on corticosteroids or any other immune-modifying medications. Subjects were recruited from the medical staff of the Department of Medicine at the participating institutions. Six apparently healthy subjects were recruited from the medical staff of the participating institutions.

Active SLE patients: Age 18–29, fulfilled the 1982 revised criteria of the American College of Rheumatology (formerly the American Rheumatism Association) for classification of SLE [33]. Patients with previous documentation of a diagnosis of a connective tissue disorder other than SLE were excluded. 18 subjects were enrolled.

Experimental conditions

When THP-1 cells had reached a density of 10^5– 10^6 cells ml^{-1}, the culture media was aspirated, and the cells were rinsed twice with Dulbecco's phosphate-buffered saline (DPBS) without calcium and magnesium. The cells were resuspended in fresh RPMI media without FBS and then incubated at 37°C in a 5% CO_2 atmosphere for 3 h before mRNA isolation and 24 h before protein isolation, in six-well plates, under the following conditions:

- **a.** Medium containing 50% human plasma from apparently healthy subjects.
- **b.** Medium containing 50% human plasma from SLE patients.
- **c.** Pre-incubation for 1 h in medium containing neutralizing antibody against IFN-γ (0.04 μg ml^{-1}, R&D Systems # MAB285 (Minneapolis, MN) followed by a 3-h incubation under conditions (a) or (b) as described above.
- **d.** Pre-incubation for 1 h in medium containing blocking antibody to the IFN-γ receptor (1.25 μg ml^{-1}, R&D Systems # AF673 (Minneapolis, MN) followed by a 3-h incubation under conditions (a) or (b) as described above.

RNA isolation and message analysis by RT-PCR

RNA was isolated using 1 ml Trizol reagent per 10^6 cells and dissolved in nuclease-free water. The quantity of total RNA from each condition was measured by absorption at 260

and 280 wavelengths using quartz cuvettes by ultraviolet spectrophotometry (Hitachi U2010 spectrophotometer).

RT-PCR was carried out in an Eppendorf Mastercycler Personal PCR thermocycler with reagents purchased from Applied Biosystems (Oakland, CA). Primers used in amplification reactions were generated by Sigma-Genosys (The Woodlands, TX).

For each RT reaction, 1 μg of total RNA was reverse transcribed using 50 units of Murine Leukemia Virus reverse transcriptase in the presence of 20 units of RNase inhibitor in a final volume of 50 μl. The reaction mixture contained 5 mM $MgCl_2$, 0.4 mM of each dNTP, and 2.5 μM oligo dT primers. The reaction mixtures were incubated at 42°C for 45 min. This was followed by heating at 95°C for 5 min and cooling to 5°C for 5 min.

Five microlitres of cDNA was taken from each RT mixture for PCR amplification using 27-hydroxylase-specific primers as well as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control primers. The 27-hydroxylase-specific primer pair spans a 311-base pair sequence encompassing nucleotides 491–802 of the human 27-hydroxylase cDNA [27, 34]. Nontemplate controls were included for each primer pair to check for significant levels of any contaminants. The PCR reaction was carried out using 1 unit of AmpliTaq DNA polymerase, 2 mM $MgCl_2$, 0.4 mM of each dNTP and 0.15 μM of the upstream and downstream primers. The PCR protocol included the following: an initial denaturation step at 94°C for 5 min; 30 cycles with a denaturation step of 1 min (for 27-hydroxylase) and 45 s (for GAPDH) at 94°C, an annealing step of 1 min at 62°C (for 27-hydroxylase) and 58°C (for GAPDH), and an extension step of 1 min at 72°C for both 27-hydroxylase and GAPDH, and a final extension step of 7 min at 72°C for both.

In all cases, equal volumes (10 μl $lane^{-1}$) of amplified PCR products were mixed with 1 μl of 6X DNA loading buffer (GIBCO BRL; Carlsbad, CA) and separated by agarose gel electrophoresis on a 1.5% agarose gel. The DNA was electrophoresed at 100 V for 30 min. The 1.5% agarose gel was stained with 0.5 μg ml^{-1} ethidium bromide to visualize the DNA.

The GAPDH controls (10 μl $lane^{-1}$) were loaded at two concentrations, 1 and 0.20 μg μl^{-1} of starting total RNA amount. The DNA samples electrophoresed in agarose gel were visualized and photographed under ultraviolet light (320 nm) using a Kodak trans-illuminator. The gel images were photo-documented, and net intensities were measured with Kodak Digital Science 1D, version 2.0.3, after imaging with Kodak Digital Science Electrophoresis Documentation and Analysis System 120. All experimental results were normalized to the mean density of GAPDH.

Protein extraction and Western blot analysis

Western blot detection of 27-hydroxylase was performed as described previously [26]. Total cell lysates were prepared for Western immunoblotting using RIPA lysis buffer (98% PBS, 1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]). 100 μl of RIPA lysis buffer and 10 μl of protease inhibitor cocktail (Sigma) were added to the cell pellet from each condition and incubated on ice for 35 min with vortexing every 5 min. Supernatants were collected after centrifuging at 10,000×*g* at 4°C for 10 min using an Eppendorf 5415C centrifuge. The quantity of protein in each supernatant was measured by absorption at 560 nm using a Hitachi U2010 spectrophotometer.

Cell lysate protein samples (20 μg $lane^{-1}$) were boiled for 5 min, loaded onto a 10% polyacrylamide gel, electrophoresed for 1.5 h at 100 V then transferred to a nitrocellulose membrane in a semi-dry transblot apparatus for 1 h at 100 V. The nitrocellulose membrane was blocked for 4 h at 4°C in blocking solution (3% nonfat dry milk dissolved in

1×Tween20-tris-buffered saline [TTBS]) then immersed in a 1:300 dilution of primary antibody (18.7 μg ml^{-1}) in blocking solution overnight at 4°C. The primary antibody is an affinity-purified rabbit polyclonal anti-peptide antibody raised against residues 15–28 of the cholesterol 27-hydroxylase protein [35]. The following day, the membrane was washed five times in TTBS for 5 min per wash then incubated at room temperature in a 1:3,000 dilution of ECL donkey anti-rabbit IgG Horseradish peroxidase-linked species-specific whole antibody (Amersham Biosciences, product Code NA934). The five washes in TTBS were repeated and then the immunoreactive protein was detected using ECL Western blotting detection reagent (Amersham Biosciences, Cat No. RPN2106) and film development in SRX-101A (Konica Minolta).

As control, on the same transferred membrane, beta-actin was detected using mouse anti-beta-actin (diluted in 1:1,000, from abCam, product Code: ab6276) and ECL sheep anti-mouse-IgG Horseradish peroxidase-linked species-specific whole antibody (diluted in 1:2,000, from Amersham Biosciences, product Code NA931) and all other similar steps as above. The stained nitrocellulose membrane was scanned with a Kodak scanner, and the net intensities were measured with Kodak Digital Science 1D, version 2.0.3 for analysis.

Macrophage foam cell transformation and staining

THP-1 human monocytes (10^6 cells ml^{-1}) in 12-well plates were treated with phorbol dibutyrate, 300nM (Sigma) for 48 h at 37°C to facilitate differentiation into macrophages. The differentiated macrophages were washed three times with PBS, then incubated in the presence of 25% SLE patient plasma or apparently healthy control human plasma (CHP) at 37°C in 5% CO_2, for 18 h. Cells were cholesterol-loaded with acetylated LDL (50 μg ml^{-1}, Intracel, Issaquah, Washington) and further incubated in RPMI1640 at 37°C, in 5% CO_2 for 48 h. Studies were performed in triplicate.

Immediately following incubation, media was aspirated and cells were fixed in the same 12-well plates used for incubation, with 4% paraformaldehyde in water, for *2*–4 min. Cells were stained with 0.2% Oil-Red-O in methanol for 1–3 min. Cells were observed via light microscope (Axiovert 25-Zeiss) with 100× magnification and then photographed using a Kodak DC 290 Zoom Digital Camera. The number of foam cells formed in each condition was calculated manually and presented as percentage foam cell formation.

Statistical analysis of experimental data

Statistical analysis was performed using SigmaStat v2.03 (SPSS Inc, Chicago, IL). Pairwise comparison was made between each treatment condition and control using student *t* test. Data are presented as the mean ± SEM.

Results

THP-1 monocytes/macrophages and HAEC exposed to lupus plasma exhibit diminished cholesterol 27-hydroxylase expression

27-Hydroxylase message decreased by 47 ± 8% ($n = 3$, $p < 0.008$) in THP-1 cells and by 51 ± 5.5% ($n = 5$, $p < 0.001$) in HAEC after a 3-h exposure to SLE plasma (Fig. 1).

Blocking the action of IFN-γ mutes the effect of SLE plasma on cholesterol 27-hydroxylase

Pre-exposure of THP-1 monocytes to IFN-γ receptor blocking antibody followed by incubation in SLE patient plasma for 3 h prevents the SLE plasma from decreasing 27-hydroxylase message (2.7 ± 0.7%) (Figs. 2, 3). THP-1 cells treated with equivalent concentrations of CHP exhibited no diminution of 27-hydroxylase message.

Changes in 27-hydroxylase message level resulted in concomitant changes in protein expression. Total protein isolated from THP-1 monocytes was subjected to Western blot analysis which confirmed a significant downregulation of 27-hydroxylase protein in cells treated with SLE patient plasma when compared to untreated controls (Fig. 4). THP-1 monocytes pre-incubated with IFN-γ receptor blocking antibody or IFN-γ neutralizing antibody demonstrated no changes in 27-hydroxylase protein level despite exposure to SLE patient plasma.

Lupus plasma increases THP-1 macrophage foam cell transformation

THP-1 macrophages were incubated 18 h in medium containing 25% CHP or lupus patient-derived plasma and cholesterol-loaded with 50 μg ml^{-1} acLDL for further 48-h incubation. Foam cell formation was quantified as percent Oil-Red-O-stained cells. Lupus plasma more than doubled transformation of acLDL-treated THP-1 macrophages into foam cells (74 ±3% vs. 35 ± 3% for CHP, $n = 3$, $p < 0.001$) (Fig. 5).

Discussion

Atherosclerosis is the result of a complex orchestration of inflammatory and immunological mechanisms [36].Critical to the atherosclerotic process is deregulation of cholesterol balance in cells of the arterial wall [37]. Immune reactants such as the cytokine IFN-γ or complement C1q-bound immune complexes can modulate the function of the protein components involved in reverse cholesterol transport in monocytes or macrophages and endothelium of the artery [23, 24, 38]. Previous and present independent clinical studies suggest that immunological derangements present in SLE patient plasma include increased levels of IFN-γ, tumor necrosis factors (TNF), interleukins (IL), and complement C1q-mediated immune complexes [39–41]. In lupus-prone murine models, enhanced activation of the immune system, elevated cytokine levels, and macrophage accumulation are associated with accelerated atherosclerosis [42]. The current findings demonstrate that the plasma of persons with the systemic inflammatory and autoimmune disease SLE is pro-atherogenic and that a likely contributor to this effect is elevated levels of circulating IFN-γ. This is in close agreement with our recent paper demonstrating that THP-1 monocytes exposed to SLE plasma overexpress CD36, an atheroma-promoting class B scavenger receptor that recognizes oxidized lipoproteins [43].

We have shown previously that IFN-γ downregulates 27-hydroxylase message and protein in HAEC and THP-1 monocytes [23, 24]. However, the effect of exposure to SLE plasma on expression of this reverse cholesterol transport protein and on foam cell transformation has not been studied. The influence of other circulating inflammatory mediators in lupus plasma on the response of HAEC and monocytoid cells to IFN-γ could not be predicted. Thus, the present study provides strong evidence that cholesterol transport is modulated in the presence of an inflammatory milieu by endogenous IFN-γ, as seen in SLE.

The critical role of IFN-γ in the development of atherosclerosis has been demonstrated in murine models [44]. Proatherogenic effects of IFN-γ include induction of VCAM-1 on endothelial cells, and lipoprotein receptors on smooth muscle cells and macrophages [45]. Apolipoprotein E knockout (ApoE KO) mice (hypercholesterolemic mice that develop atherosclerosis) crossed with IFN-γ receptor KO mice display reduced lesion size, lipid accumulation, and cellularity [44]. ApoE KO mice given IFN-γ exhibit a twofold increase in atherosclerotic lesion size in the ascending aorta compared to controls [44, 46].

The 27-hydroxylase is a key enzyme involved in the extrahepatic metabolism of cholesterol. It oxygenates cholesterol into oxysterols, mainly 27-hydroxycholesterol, and facilitates reverse cholesterol transport of excess cholesterol back to the liver efficiently for

metabolism to bile [32]. Human arterial endothelium, monocytes/macrophages, and THP-1 monocytes express high levels of 27-hydroxylase [23, 27, 47]. The enzyme is involved in clearing cellular cholesterol load, impeding the transformation of cholesterol-laden macrophages into pro-atherogenic foam cells [24, 48]. Here, we report marked downregulation of the anti-atherogenic 27-hydroxylase in THP-1 human monocytes and HAEC upon exposure to SLE patient plasma. Masking of IFN-γ receptors on the THP-1 cell surface negates the effect of SLE patient plasma on 27-hydroxylase expression at both message and protein levels. We demonstrated previously that IFN-γ acting through its receptors decreased 27-hydroxylase expression and increased rate of foam cell formation significantly in cholesterol-loaded THP-1 macrophages [24, 49]. The accumulated data indicate that the elevated level of IFN-γ present in SLE patient plasma is involved in modulating expression of 27-hydroxylase in THP-1 cells and may contribute to increased atherogenic risk in lupus patients in vivo.

The plasma of lupus patients is known to have atherogenic properties [50] and our laboratory recently reported that exposure of THP-1 monocytes/macrophages to lupus plasma causes marked elevation in the level of the CD36 scavenger receptor responsible for uptake of oxidized lipids [42]. There are a multitude of factors that may contribute to the atherogenic nature of lupus plasma. These include autoantibodies, immune complexes, cytokines, chemotactic and thrombogenic factors, and enhanced lipoprotein oxidation [38, 51].

The present work has a number of limitations. It is a small-scale observational study that suggests a role for IFN-γ in dysregulating cholesterol outflow in lupus, providing one aspect of a biochemical rationale for the high incidence of premature coronary artery disease persistently observed in these patients. The investigators who quantitated the 27-hydroxylase expression were blinded to the diagnosis of the subjects whose plasma was being used. Individual patient records were not available for review, so we were unable to adjust the analyses for subject and environmental differences and co-morbidities that might be confounders. We were unable to assess the relative contribution of immune complexes because the plasma samples were frozen and cold-precipitable immune complexes would have been precipitated out.

This study is consistent with current knowledge of the association between SLE and atheroma development. Our findings point to questions that need to be addressed in future studies. Enrollment is currently ongoing in a larger study that will pinpoint specific SLE plasma fractions responsible for atherogenic effects. Relative potency of plasma from individuals with SLE in disrupting reverse cholesterol transport may also have predictive value in identifying patients most vulnerable to cardiovascular complications of SLE.

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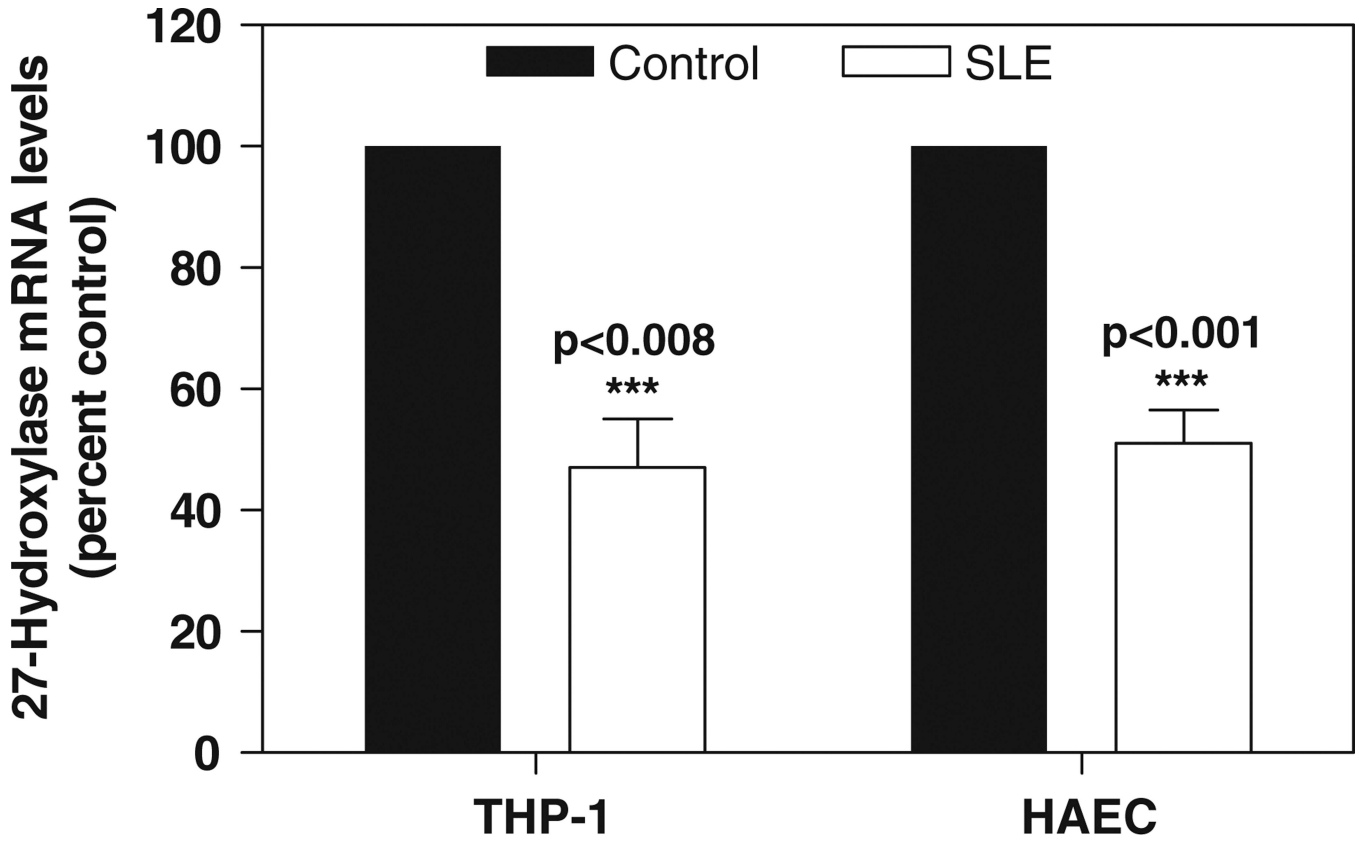

Fig. 1.

Effect of SLE patient plasma on cholesterol 27-hydroxylase mRNA expression in THP-1 and HAEC. Cultured THP-1 cells and HAEC were exposed to 50% CHP or 50% plasma from SLE patients for 3 h. Quantitative analysis for changes in 27-hydroxylase expression was performed using RT-PCR with GAPDH message as an internal standard from isolated total RNA

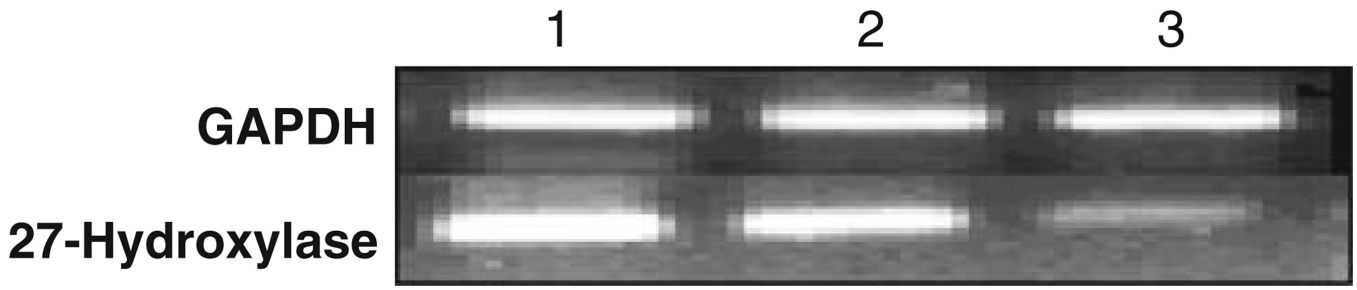

Fig. 2.

Impact of IFN-γ receptor blockade on downregulation of cholesterol 27-hydroxylase message in THP-1 monocytes by SLE patient plasma. Cultured THP-1 cells were untreated or exposed to 50% plasma from SLE patients under the following conditions: *lane 1* control, untreated THP-1 cells only in RPMI1640 media; *lane 2* THP-1 cells pre-incubated with IFN-γ receptor blocking antibody (1.25 μg ml^{-1}) followed by a 3-h incubation in 50% SLE patient plasma/50% RPMI1640 media; *lane 3* THP-1 cells in 50% SLE patient plasma/50% RPMI1640 media after 3 h incubation. Total RNA isolated from cells exposed to each condition was reverse transcribed and amplified by PCR with GAPDH message as an internal standard

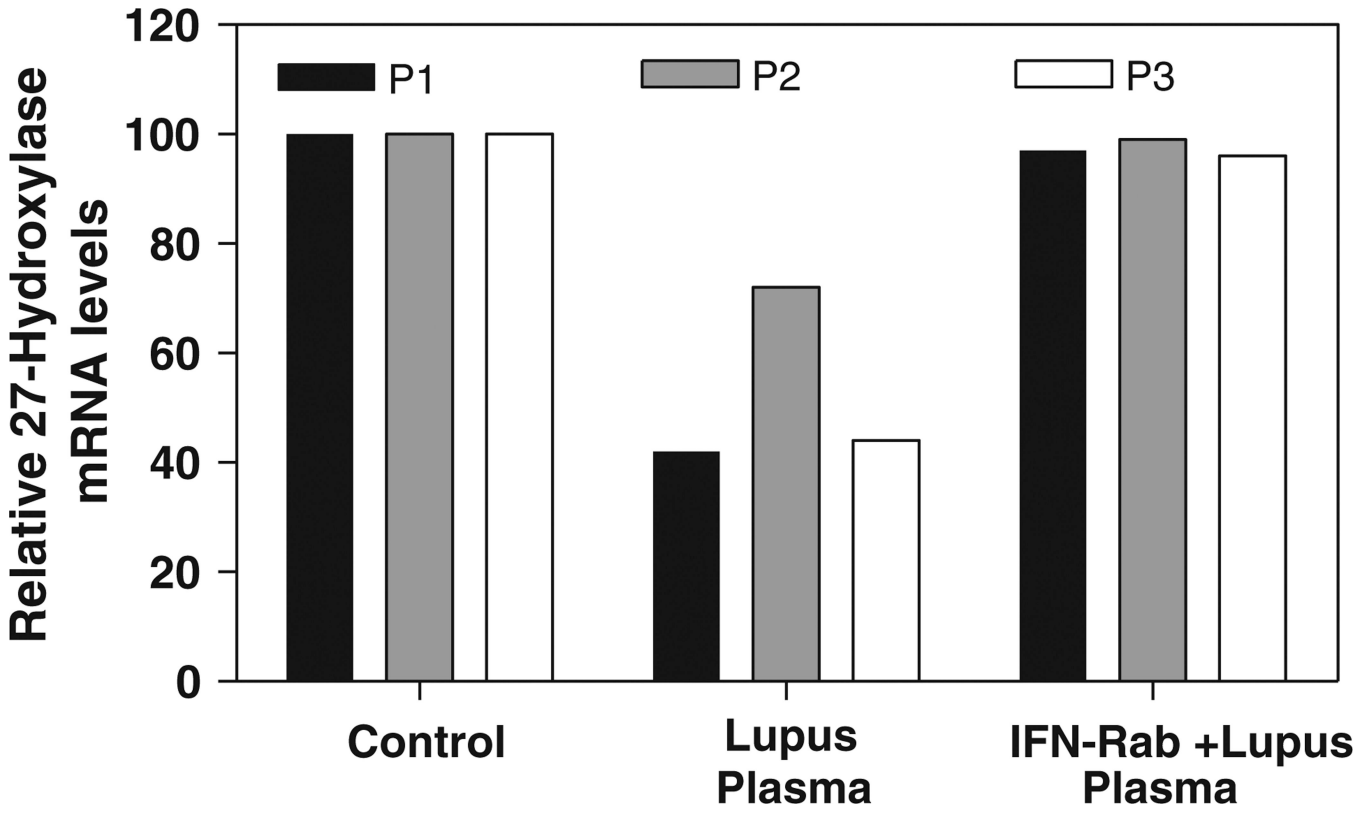

Fig. 3.

QRT-PCR analysis of 27-hydroxylase message modulation in THP-1 cells by SLE patient plasma. Quantitative analysis for 27-hydroxylase message was performed in the presence of plasma from three individual SLE patients in the absence or presence of IFN-γ receptor blockade. *Control* untreated THP-1 cells in RPMI1640 media; *PS* THP-1 cells incubated with 50% SLE patient plasma/50% RPMI1640 media for 3 h; *IFN-Rab* THP-1 cells pre-incubated for 1 h with IFN-γ receptor blocking antibody (1.25 μg ml^{-1}) followed by a 3-h incubation in 50% SLE patient plasma/50% RPMI1640 media

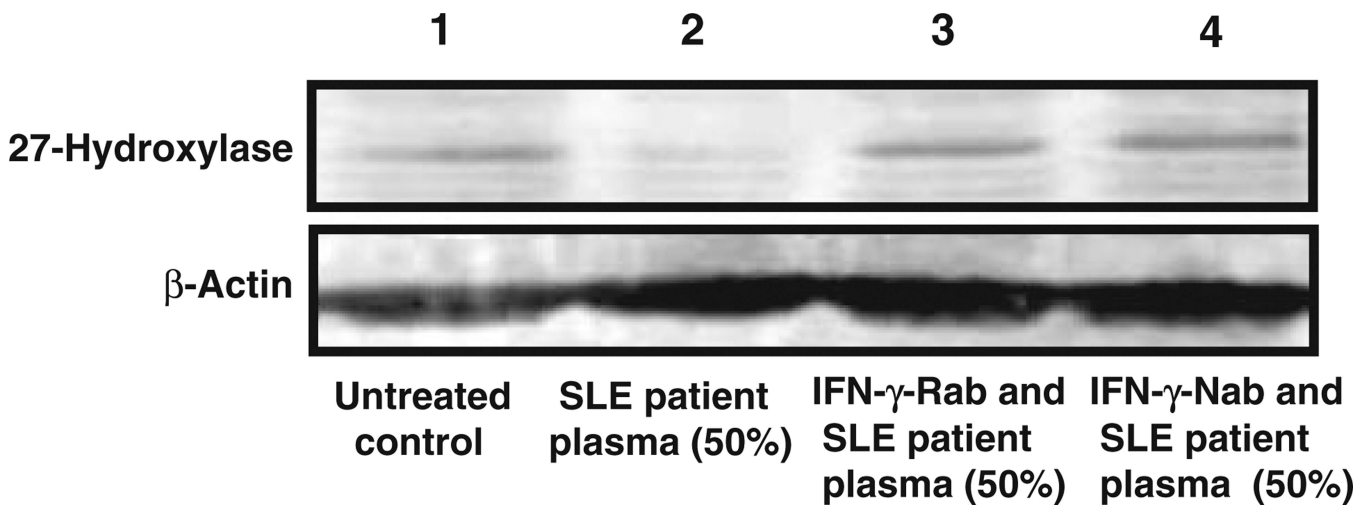

Fig. 4.

IFN-γ neutralizing and IFN-γ receptor blocking antibodies abolish SLE patient plasma-mediated downregulation of 27-hydroxylase protein in THP-1 monocytes. Cultured THP-1 cells were untreated or exposed to 50% plasma from SLE patients under the following conditions: *lane 1* control untreated THP-1 cells; *lane 2* THP-1 cells in 50% SLE patient plasma/50% RPMI1640 media; *lane 3* THP-1 cells pre-incubated for 1 h with IFN-γ receptor blocking antibody (1.25 g ml^{-1}) followed by exposure to 50% of SLE patient plasma/ 50% RPMI1640 media; *lane 4* SLE patient plasma pre-incubated for 1 h with IFN-γ neutralizing antibody (1.25 g ml^{-1}) prior to incubation with THP-1 cells in RPMI1640 media. Following a 24-h incubation, total cellular protein was isolated and run on an SDS-polyacrylamide gel and immunoblotted with human 27-hydroxylase-specific polyclonal antibody

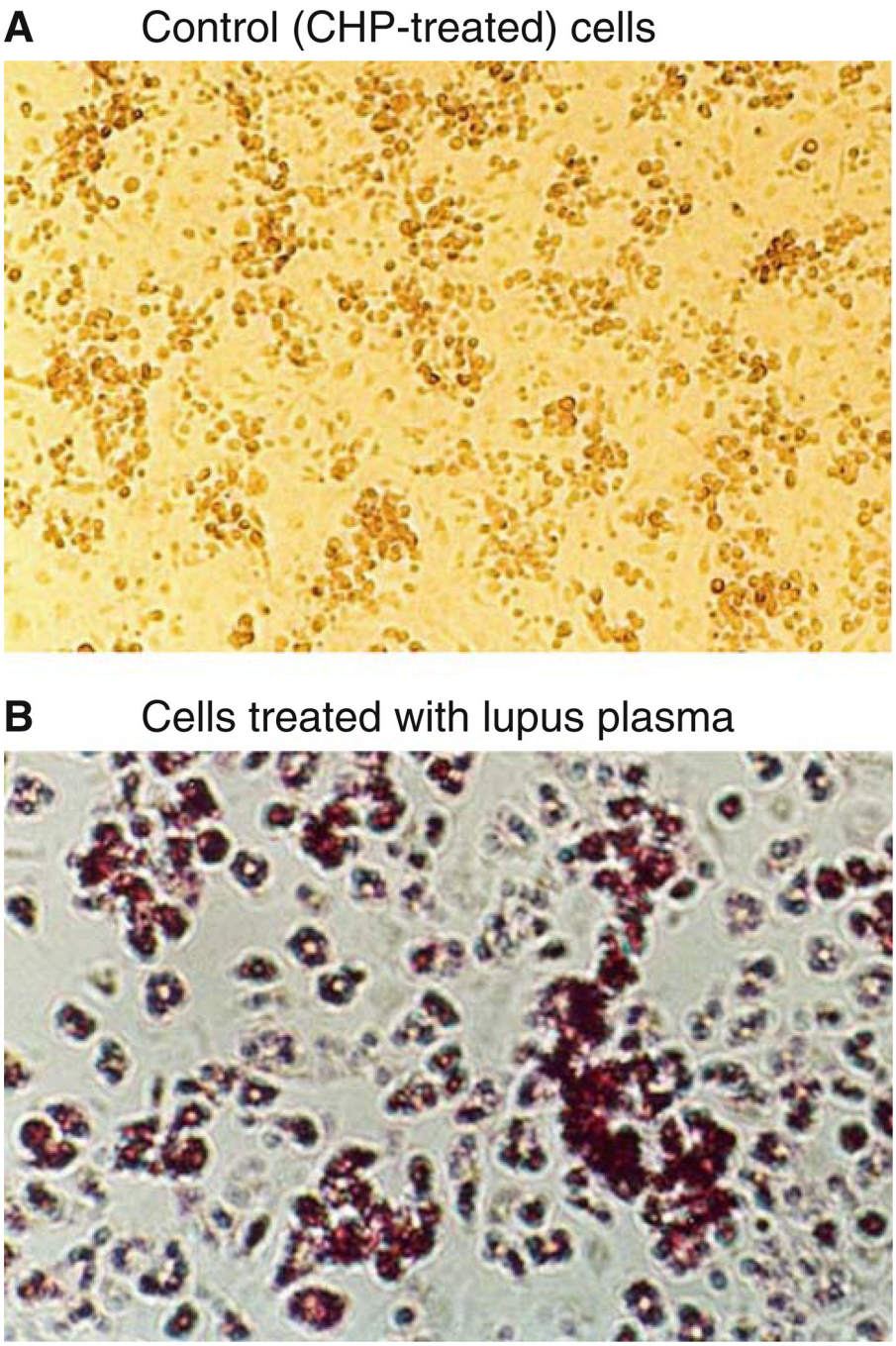

Fig. 5.

Exposure to SLE patient plasma increases foam cell formation in THP-1 macrophages. THP-1 differentiated macrophages were incubated for 18 h in media containing 25% CHP or 25% SLE patient plasma. Macrophages were then treated with acLDL (50 μg ml^{-1}) and incubated for an additional 48 h. Representative photomicrographs of Oil-Red-O staining to detect foam cells