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CHOLESTEROL 27-HYDROXYLASE BUT NOT APOLIPOPROTEIN apoE CONTRIBUTES TO A_{2A} ADENOSINE RECEPTOR STIMULATED REVERSE CHOLESTEROL TRANSPORT

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Abstract

Movement of free cholesterol between the cellular compartment and acceptor is governed by cholesterol gradients that are determined by several enzymes and reverse cholesterol transport proteins. We have previously demonstrated that adenosine A_{2A} receptors inhibit foam cell formation and stimulate production of cholesterol 27-hydroxylase (CYP27A1), an enzyme

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CONFLICT OF INTEREST

Taiese C. Bingham, PhD: none

Bruce N. Cronstein, MD:

Intellectual Property:

Patents on use of adenosine A_{2A} receptor agonists to promote wound healing and use of A_{2A} receptor antagonists to inhibit fibrosis.

Patent on use of adenosine A₁ receptor antagonists to treat osteoporosis and other diseases of bone.

Patent on the use of adenosine A₁ and A_{2B} Receptor antagonists to treat fatty liver

Patent on the use of adenosine A_{2A} receptor agonists to prevent prosthesis loosening

Consultant (within the past two years), all <\$10,000:

Cypress Bioscience, Inc.

King Pharmaceutical (licensee of patents above)

CanFite Biopharmaceuticals

Bristol-Myers Squibb

Cellzome

Tap Pharmaceuticals

Prometheus Laboratories

Regeneron (Westat, DSMB)

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Endocyte

Protalex

Allos, Inc.

Combinatorx

Kyowa Hakka

Hoffman-LaRoche

Savient

Avidimer Therapeutics

Stock

CanFite Biopharmaceuticals received for membership in Scientific Advisory Board.

Bagels, ice cream, snacks, etc

Eli Lilly & Co.

UCB

Pfizer

involved in the conversion of cholesterol to oxysterols. We therefore asked whether the effect of adenosine A_{2A} receptors on foam cell formation *in vitro* are mediated by CYP27A1 or apoE, a carrier for cholesterol in the serum. We found that specific lentiviral siRNA infection markedly reduced apoE or 27-hydroxylase mRNA in THP-1 cells. Despite diminished apoE expression ($p < 0.0002$, IFN γ CGS vs. IFN γ alone, $n = 4$) CGS-21680, an adenosine A_{2A} receptor agonist, inhibits foam cell formation. In contrast, CGS-21680 had no effect on reducing foam cell formation in CYP27A1 KD cells ($4 \pm 2\%$ $p < 0.5113$ inhibition vs. IFN γ alone $n = 4$). Previously we reported the A_{2A} agonist CGS-21680 increases apoAI-mediated cholesterol efflux nearly 2-fold in wildtype macrophages. Adenosine receptor activation had no effect on cholesterol efflux in CYP27A1 KD cells but reduced efflux in apoE KD cells. These results demonstrate that adenosine A_{2A} receptor occupancy diminishes foam cell formation by increasing expression and function of CYP27A1.

Keywords

Atherosclerosis; Inflammation; Adenosine; Macrophages

INTRODUCTION

Previously we have reported that adenosine A_{2A} receptor activation stimulates enhanced ABCA1-dependent cholesterol efflux but not ABCG1-dependent cholesterol efflux from human macrophages [1–5]. Cholesterol efflux from these cells contributes, *in vivo*, to reverse cholesterol transport from the periphery to the liver for elimination in the bile. However, in addition to the cholesterol transporters ABCA1 and ABCG1 there are several other proteins that contribute to reverse cholesterol transport, including apoprotein apoE and the enzyme cholesterol 27-hydroxylase (CYP27A1)[6].

CYP27A1 contributes to enhanced cholesterol efflux from cells and leads to the transport of more soluble oxysterols to the liver for elimination [7]. In lipid-laden macrophages cholesterol is converted by CYP27A1 to 27-hydroxycholesterol which is more soluble in aqueous media [7]. The cytochrome P450 enzyme 27-hydroxylase has a number of roles in maintaining lipid homeostasis, however its main role is promote the formation of bile acids which help promote absorption of dietary lipids in the intestine and to stimulate the biliary excretion of cholesterol (Norlin et al, 2007). Point mutations in the human CYP27A1 gene are found in patients with the inherited lipid storage disease Cerebrotendinous Xanthomatosis, a syndrome characterized by accumulation of bile acids, accelerated atherosclerosis, osteoporosis, and neurologic impairment [8].

The cholesterol transporter apoE mediates numerous effects which result in protection from atherosclerosis. apoE promotes efficient uptake of triglyceride-rich lipoproteins from circulation, maintains normal macrophage lipid homeostasis, plays a role in cellular cholesterol efflux and reverse cholesterol transport, acts as an antioxidant, inhibits platelet aggregation, and modulates immune function [9]. apoE is produced in both hepatic cells and macrophages and is primarily involved in hepatic uptake of lipoprotein particles, stimulation of cholesterol efflux from macrophages, and regulation of immune and inflammatory responses [10]. In mice disruption of the apoE gene results in spontaneous atherosclerosis. In apoE knockout mice restoration of apoE prevents the atherosclerotic phenotype, providing further strong support for a direct role of this transporter in prevention of atherosclerosis. Also, apoE recycling requires the docking of HDL/apoA-I to receptors, such as ABCA1 and ABCG1, which then facilitates internalization of HDL/apoA-I [10]. The docking of HDL/apoA-I to the membrane promotes cholesterol translocation from the intracellular cholesterol pool to the cell membrane through the activation of phospholipases and protein kinase C [11,12]. It has also been found that the nuclear hormone receptor LXR

induces apoE expression in macrophages and ABCA1 accelerated apoE secretion from macrophages [12].

Because both CYP27A1 and apoE play such critical roles in reverse cholesterol transport and prevention of atherosclerosis we determined whether activation of the adenosine A_{2A} receptor diminished foam cell formation and promoted reverse cholesterol transport via enhanced expression of these two proteins. We found that adenosine A_{2A} receptor stimulation inhibited foam cell formation by a mechanism dependent on the expression of CYP27A1.

MATERIALS AND METHODS

Infection of THP-1 cells

THP-1 cells suspended in medium (1×10^6 /ml) are treated with Hexadimethrine Bromide from Sigma Chemical Co. (St. Louis, MO) (8 ug/ml, final) and 10^8 transducing units of lentiviral particles corresponding to human apoE shRNA (SHVRS 08090813MN), human CYP27A (SHVRS 09300804MN) or scrambled shRNA (SHC002V 05210717MN) with a puromycin selection marker (Sigma Aldrich, St. Louis). Media is changed 24 hours following infection and replaced with medium containing puromycin (1ug/ml). After 7 days cells are isolated and resuspended in medium, some cells were lysed and assayed for mRNA for apoE before and after treatment with IFN γ in the presence or absence of the A_{2A}-selective agonist CGS-21680, the selective antagonist ZM-241385 or their combination (all at 1 μ M final concentration).

Isolation of mRNA for Real Time RT-PCR

As we have previously described [1], apoE KD cells or CYP27A1 KD cells (10^6 cells/ml) are treated with PMA (100nM) phorbol 12-myristate 13-acetate for 48 h (37°C, 5% CO₂) to differentiate into macrophages. Cells are washed with serum-free RPMI 1640 and further incubated with 50 ug/ml acetylated LDL for 3 h in the presence of IFN γ (500U/ml) in the presence or absence of CGS21680 (1 μ M), the A_{2A} antagonist ZM-241385 (1 μ M) or their combination. Total RNA from cells is then extracted using Trizol (Invitrogen) according to the manufacturer's instructions, and 5 μ g total RNA is used for first-strand, complementary cDNA synthesis primed with random hexamer primers using an RNA polymerase chain reaction (PCR) core kit (Invitrogen,Carlsbad,CA).

Quantitative real-time PCR

For quantification of apoE message by real-time PCR, the following primer pairs are used: (apoE) 5'-aggatctacgcaaccgactc-3', 5'-ggcgtatcatgtctccacta-3', (CYP27A1) 5'-tgccgccagctctgaaccag-3', 5'-tccactggggaggaaggtg-3'. Human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression is used as an internal standard (5'-aacatcatccctgcctctac-3', 5'-ccctgttgctgtagccaaa-3') under parallel conditions. PCR amplifications are detected using the SYBR Green kit from Applied Biosystems [1].

Foam cell formation and staining

apoE KD or CYP27A1 THP-1 human monocytes (10^6 cells/ml) in 4 well glass chamber slides are treated with 100 nM phorbol 12-myristate 13-acetate, PMA, (Sigma Aldrich, St. Louis) for 2 days at 37°C to stimulate differentiation into macrophages. Macrophages are washed three times with phosphate-buffered saline (PBS) and further incubated in RPMI 1640 (37°C, 5% CO₂) for 24 hr under the following conditions: a) acetylated low-density lipoprotein (50 μ g/ml, Intracel, Frederick, MD) plus IFN γ (500U/ml) in the presence or absence of adenosine A_{2A} receptor agonist CGS-21680 (1 μ M) or the selective adenosine A_{2A} receptor antagonist ZM-241385 (1 μ M). Following incubation media is aspirated and

slides are washed with PBS and fixed in 10% formalin (Sigma Aldrich, St. Louis) for 10 minutes. Cells are washed in distilled water rinsed in 60% isopropanol and stained with 0.2% Oil Red O (Sigma Aldrich, St. Louis) for 15 minutes. Cells are rinsed again with 60% isopropanol and dipped into haematoxylin to stain for cell nuclei. Cells are briefly washed with distilled water and cover slips are mounted on slides using glycerine jelly. Foam cells, recognized as macrophages stained with Oil Red O, are visualized via light microscope (Olympus 820) with 100 $\times$ magnification and then photographed using a Kodak DC 290 Zoom Digital Camera. The number of foam cells formed in each condition are calculated manually and presented as a percentage foam cell formation [1].

AcLDL uptake by THP-1 Cells

ApoE KD or CYP27A1 THP-1 cells are treated as above in 12-well plates washed once with PBS. Cells are then incubated in Ham's F-12 supplemented with PSG and 2% lipoprotein-deficient fetal calf serum (LPD-FCS; Sigma) in the presence or absence of 5 μ g/ml Dil (1,1'-dioctadecyl-1-3,3,3',3'-tetramethylindocarbocyanine perchlorate) labeled Alexa-Fluor AcLDL (Invitrogen) for 3 h at 37°C. Cells are washed five times in PBS and fluorescence is measured using the Perkin Elmer V³ Multilabel Counter (485 nm/535nm 0.1s) [1].

Cholesterol Efflux from THP-1 Cells

apoE KD or CYP27A1 macrophages are maintained in RPMI 1640 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C, 5% CO₂. Cells are incubated at 10⁶ cells/ml in the presence of PMA for 4 days in order to induce differentiation into macrophages. apoE KD or CYP27A1 KD macrophages are labeled with ³H-cholesterol (0.2 μ Ci/ml) for 24h (37°C, 5% CO₂). The cells are washed 2 times with serum-free medium containing 0.2% BSA and then cultured overnight in medium. To measure the effect of adenosine receptor agonists/antagonists on cholesterol efflux, cells are incubated for 24 h with 50 μ g/ml apoA1 in the absence or presence of CGS21680 (1 μ g/ml), ZM-241385 (1 μ g/ml) or their combination and IFN γ . At the end of the incubation, cells are centrifuged (10,000 G for 5 min) to remove medium and then lysed. Aliquots (200 μ l) from the medium and cells are counted separately using liquid scintillation counting [1].

Statistics

Reported as the mean $\pm$ SEM, differences between treatment groups were analyzed by using one-way ANOVA followed by unpaired Students T-test to evaluate levels of significance. P<0.05 was accepted as being statistically significant. All statistical analyses are performed with SigmaStat software v. 3.0 (SPSS, Inc. Chicago, IL).

RESULTS

Lentiviral constructs reduce apoE and CYP27A1 mRNA in human THP-1 cells

We have previously shown that adenosine acting at A_{2A} receptors stimulates ABCA1-dependent cholesterol efflux but not ABCG1-dependent cholesterol efflux [1]. In order to further probe the mechanism by which adenosine receptor activation enhances cholesterol efflux from macrophages and diminishes foam cell formation we diminished expression of other molecules involved in reverse cholesterol transport. Using siRNA technology, we were able to reduce apoE mRNA expression (p< 0.0001, apoE siRNA vs. scrambled siRNA, n=3) and apoE protein expression (Fig. 1). We also reduced 27-hydroxylase mRNA expression (p< 0.0001, CYP27A1 siRNA vs. scrambled siRNA, n=3) and CYP27A1 protein expression (p< 0.01, CYP27A1 siRNA vs. control n= 3) in order to more accurately access adenosine receptor stimulation on reverse cholesterol transport (Fig. 2).

A_{2A}R agonist CGS21680 has no effect on apoE mRNA levels

IFN- γ increases foam cell formation and decreases ABCA1 mRNAs and ABCA1 protein levels in human THP-1 cells. The selective A_{2A}R agonist CGS-21680 reverses the effects of IFN γ on ABCA1 and increase ABCA1 mRNA of CYP27A1 and ABCA1 in THP-1 monocytoïd cells and in murine monocytes (Reiss et al., 2004). In contrast, neither IFN-g nor CGS21680 significantly affects expression of mRNA for apoE (Fig. 3).

Adenosine A_{2A} receptor occupancy blocks IFN- γ -mediated foam cell formation in apoE KD cells but not in CYP27A1 KD cells

Adenosine A_{2A} receptor activation stimulates cholesterol efflux from and diminishes foam cell formation by macrophages formed from THP-1 cells by a mechanism dependent on ABCA1 expression. To more fully understand how adenosine A_{2A} receptor stimulation promotes cholesterol efflux and inhibits foam cell formation we determined whether the adenosine A_{2A} receptor agonist CGS-21680 (1 μ M) inhibits foam cell formation in apoE KD cells. We found that the loss of apoE expression did not affect the capacity of CGS21680 to inhibit foam cell formation (Fig. 4), a phenomenon completely reversed by the A_{2A}R antagonist ZM-41385. However, the adenosine A_{2A} receptor agonist CGS-21680 lost the capacity to diminish foam cell formation in CYP27A1 KD cells (Fig.5). This surprising observation is consistent with the hypothesis that adenosine receptor-mediated stimulation of CYP27A1 expression also plays a role in inhibiting foam cell formation by an apoE-independent mechanism.

Cholesterol uptake is unaffected by adenosine receptor activation in apoE KD and CYP27A1 macrophages

Because cholesterol is actively taken up by macrophages during foam cell formation and requires the docking of HDL/apoA-I to receptors such as ABCA1 and ABCG1 we determined whether the A_{2A} receptor agonist CGS-21680 decreased foam cell formation by inhibiting cholesterol uptake. We found that the A_{2A} receptor agonist CGS-21680 did not diminish cholesterol uptake in either apoE or CYP27A1 KD cells (Fig. 6A–B). These findings further suggest the hypothesis that CGS-21680 does not decrease foam cell formation by inhibiting cholesterol uptake but by enhancing cholesterol efflux in an ABCA1 and CYP27A1 dependent manner.

Selective A_{2A}R agonist CGS-21680 increases apoAI -mediated cholesterol efflux in apoE KD but not in CYP27A1 KD cells

ATP Binding Cassette Transporters ABCA1 and ABCG1 mediate cholesterol efflux in the presence of the acceptor molecules apoAI and HDL, respectively. Earlier we reported that adenosine A_{2A} receptor ligation inhibits foam cell formation and increases cholesterol efflux without affecting cholesterol uptake by a mechanism that depends on ABCA1- but not ABCG1-dependent cholesterol efflux. Because loss of CYP27A1 expression also abrogates the adenosine A_{2A} receptor-dependent inhibition of foam cell formation we tested the capacity of A_{2A} receptor stimulation to enhance apoE-dependent or CYP27A1 dependent cholesterol efflux. The A_{2A}R agonist CGS-21680 significantly increased apoAI mediated [³H]-cholesterol efflux (from 10.0 $\pm$ 2% to 17.5 $\pm$ 2% ³H-cholesterol efflux, $p < 0.0300$ vs. control, $n=3$) in IFN- γ treated apoE KD macrophages but not in CYP27A1 KD cells (Fig. 7A–B). This finding further corroborates the hypothesis that adenosine receptor activation increases cholesterol efflux by a CYP27A1-dependent mechanism which is associated with diminished foam cell formation.

ABCA1 protein levels are unaffected in CYP27A1 KD cells treated with adenosine receptor agonist CGS-21680

We have shown that ATP Binding Cassette Transport protein ABCA1 is required for adenosine receptor mediated cholesterol transport and that A_{2A} receptor stimulation decreases foam cell formation and increases expression of ABCA1 and CYP27A1 [13]. Previous reports indicate that oxysterols, potentially produced by CYP27A1, are critical for stimulation of ABCA1 expression [14]. To determine whether the effects of adenosine A_{2A} receptor-mediated promotion of cholesterol efflux and inhibition of foam cell formation result from the indirect increase in expression of ABCA1 we determined whether CGS21680 regulated ABCA1 expression in the absence of CYP27A1 expression. We found that in CYP27A1 KD cells adenosine receptor agonist CGS-21680 did not lose its capacity to stimulate ABCA1 protein expression ($p < 0.0001$, IFN- γ CGS vs. control, $n = 4$) (Fig. 8). This finding suggests that adenosine receptor activation decreases foam cell formation and increases CYP27A1 and ABCA1-dependent cholesterol efflux through two independent mechanisms.

DISCUSSION

Here we report that adenosine A_{2A} receptor stimulation promotes cholesterol efflux and inhibits foam cell formation by a mechanism dependent on expression of CYP27A1. We have previously demonstrated that adenosine A_{2A} receptor stimulation promotes cholesterol efflux and inhibits foam cell formation by a mechanism dependent on ABCA1. It is likely that *in vivo* these mechanisms are complementary.

Both apoE and CYP27A1 play important roles in regulating cholesterol transport. In the case of apoE activation, chylomicrons and liver-derived very low-density lipoproteins (VLDL) are primarily responsible for the transport of lipids from extrahepatic cells to the liver. Ultimately these triglyceride-rich lipoproteins become hydrolyzed leading to the formation of triglyceride-rich lipoprotein remnants [15]. Subsequently hydrolyzed remnants become enriched with high-density lipoprotein (HDL)-derived apoE. apoE plays a critical role in facilitating the clearance of chylomicrons, remnant particles, and VLDL and is a major component of several classes of plasma lipoproteins [16]. apoE has been implicated in maintenance of overall plasma cholesterol homeostasis by facilitating the binding to LDL Receptors on hepatic cells, stimulating cholesterol efflux from macrophages, preventing platelet aggregation, and inhibiting proliferation of T-lymphocytes and endothelial cells [10].

The enzyme CYP27A1 belongs to a family of cytochrome P450 enzymes involved in regulating the formation of bile acids [8]. It plays a role in maintaining cholesterol homeostasis by catalyzing the conversion of cholesterol to oxysterols which activate ABCA1 transcription leading to increased cholesterol efflux [17]. It is also important to note that dysregulation of CYP27A1 leads to accelerated atherosclerosis and has been implicated in the advanced atherosclerosis that occurs prematurely in patients with lipid storage disease [8]. Our aim was to see whether activating the Adenosine A_{2A} Receptor would lead to an increase in enhanced apoE or CYP27A1-dependent cholesterol efflux.

In a previous study we focused on two ATP Binding Cassette Transporter genes known to be involved in reverse cholesterol transport, ABCA1 and ABCG1 [1]. We tested whether activation of Adenosine A_{2A} Receptor activation had any influence on ABCA1 or ABCG1 expression or function. We found that Adenosine A_{2A} Receptor activation did in fact influence ABCA1 expression and ABCA1-dependent cholesterol efflux but not ABCG1 expression or ABCG1-dependent cholesterol efflux. Because apolipoproteins and enzymes also regulate cholesterol homeostasis and transport from macrophages we also determined

whether Adenosine A_{2A} Receptor activation had any effect on apoE or CYP27A1 expression or function. We found that Adenosine A_{2A} Receptor activation had no effect on apoE mRNA levels. Also, in apoE KD cells Adenosine A_{2A} Receptor agonist CGS-21680 was able to decrease foam cell formation as was the case in wildtype and ABCG1 KD macrophages as reported previously. However CGS-21680 was not able to decrease foam cell formation in CYP27A1 KD cells. In apoE and CYP27A1 KD cells Adenosine A_{2A} Receptor agonist CGS-21680 did not influence cholesterol uptake. In apoE KD cells CGS-21690 increased cholesterol efflux but was not able to increase cholesterol efflux in CYP27A1 KD cells. The data reported from apoE KD and CYP27A1 KD cells corroborate the claim that adenosine A_{2A} receptor activation enhances cholesterol efflux by ABCA1 and CYP27A1 dependent mechanisms. Cholesterol transport proteins such as ABCG1 and apoE are not influenced by adenosine receptor signaling.

List of Abbreviations

IFNγ	Interferon-gamma
A_{2A}AR	Adenosine A _{2A} Receptor
ABCA1	ATP Binding Cassette Transporter A1
ABCG1	ATP Binding Cassette Transporter G1
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
apoA1	apolipoprotein A1
CYP27A1	27-hydroxylase

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Figure 1. Lentiviral constructs reduce CYP27A1 in human THP-1 cells

(A) Monocytes were transduced by lentivirus with shRNA for CYP27A1. mRNA was collected after 7 days of puromycin selection (10 μ g/ml) and quantitative RT-PCR was performed. Data are presented as means $\pm$ SEM (n=3). RT-PCR results are representative of three experiments and compared with those in macrophages transduced with lentiviral scramble. (B) Protein levels were quantified by calculating the average band intensity of CYP27A1/band intensity B-actin.

Figure 2. Lentiviral constructs reduce apoE in human THP-1 cells

(A) Monocytes were transduced by lentivirus with shRNA for apoE. mRNA was collected after 7 days of puromycin selection (10 μ g/ml) and quantitative RT-PCR was performed. RT-PCR results are representative of three experiments and compared with those in macrophages transduced with lentiviral scramble. Data are presented as means $\pm$ SEM. (B) Protein levels were quantified by calculating the average band intensity of apoE/band intensity B-actin.

Figure 3. A_{2A} Adenosine Receptor agonist CGS-21680 has no effect on apoE mRNA expression
 THP-1 cells (106/ml) in suspension were incubated for 3 h (37°, 5% CO₂) with or without IFN- γ (500U/ml), A_{2A} adenosine receptor agonist CGS-21680 (1 μ M) and A_{2A} adenosine receptor antagonist ZM-241385 (1 μ M). Total RNA was analyzed for apoE by real-time PCR and normalized to GAPDH. Gene expression levels were graphed as relative mRNA (% of control).

Figure 4. A_{2A} Adenosine Receptor Agonist CGS-21680 decreases IFN- γ stimulated foam cell formation in apoE KD THP-1 cells

apoE KD were incubated with PMA (10^6 cells/ml) for 48 h. Cells were then incubated with acLDL ($50\mu\text{g/ml}$) +/- pro-inflammatory cytokine IFN- γ (500U/ml), selective A_{2A} adenosine receptor agonist CGS-21680 ($1\mu\text{M/ml}$), and +/- selective A_{2A} antagonist ZM-241385 ($1\mu\text{M/ml}$) for an additional 24 h. Oil Red O stain was used to visualize lipid droplets in THP-1/macrophages. Foam cells were quantified and are expressed as percentage foam cells divided by the total cells per chamber.

Figure 5. A_{2A} Adenosine Receptor Agonist CGS-21680 does not decrease IFN- γ stimulated foam cell formation in CYP27A1 KD THP-1 cells

CYP27A1 KD cells were incubated with PMA (10^6 cells/ml) for 48 h. Cells were then incubated with acLDL ($50\mu\text{g/ml}$) +/- pro-inflammatory cytokine IFN- γ (500U/ml), selective A_{2A} adenosine receptor agonist CGS-21680 ($1\mu\text{M/ml}$), and +/- selective A_{2A} antagonist ZM-241385 ($1\mu\text{M/ml}$) for an additional 24 h. Oil Red O stain was used to visualize lipid droplets in THP-1/macrophages. Foam cells were quantified and are expressed as percentage foam cells divided by the total cells per chamber.

Figure 6. A_{2A} Adenosine Receptor Agonist CGS-21680 has no effect on cholesterol uptake in apoE KD or in 27-hydroxylase KD cells
 apoE KD (A) or CYP27A1 KD (B) macrophages were incubated in Ham's 12 medium (37°C, 5% CO₂) in the presence of AlexaFluor acLDL (acetyl-LDL; 5 μ g/ml) with or without IFN- γ (500U/ml) and A_{2A} adenosine receptor agonist CGS-21690 (1 μ M) for 3 h. Cells were washed 5 $\times$ with PBS and fluorescence intensity was quantified (435 nm/535nm) in the Victor³V multilabel counter. Values are means of three experiments $\pm$ SEM.

Figure 7. CGS-21680 increases cholesterol efflux in apoE KD cells but not in CYP27A1 KD cells
 ApoE KD (A) or CYP27A1 (B) macrophages were incubated for 24 h with or without IFN- γ (500U/ml) and A_{2A} adenosine receptor agonist CGS-21690 (1 μ M). [³H] cholesterol efflux was measured after 4 h incubations with the appropriate cholesterol acceptor, apoA1 (50ug/ml). Values are the mean of triplicates.

ABCA1

B-actin

Figure 8. CGS-21680 increases ABCA1 protein in CYP27A1 KD cells

CYP27A1 KD macrophages were incubated with IFN- γ (500U/ml), CGS-21680 (1 μ M), or IFN- γ (500U/ml) and CGS-21680 (1 μ M) for 24h. Protein levels were quantified by calculating the average band intensity of 27-hydroxylase/band intensity B-actin.