From Macrophage Interleukin-13 Receptor to Foam Cell Formation

MECHANISMS FOR $\alpha_M\beta_2$ INTEGRIN INTERFERENCE$^{*\#}$

Valentin P. Yakubenko, Linda C. Hsi, Martha K. Cathcart, and Ashish Bhattacharjee

From the Department of Cell Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195 and the Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, Ohio 44195

Background: CD36, a macrophage receptor critical for oxidized lipid uptake during atherogenesis, is up-regulated after IL-4/IL-13 stimulation.

Results: The mechanism whereby integrin $\alpha_M\beta_2$ inhibits CD36 gene expression and related foam-cell formation is elucidated.

Conclusion: Activated integrin $\alpha_M\beta_2$ suppresses CD36-mediated lipid uptake by blocking Tyk2- and Jak2-mediated signaling pathways.

Significance: $\alpha_M\beta_2$ integrin regulation of CD36 expression and function reveals a new mechanism that may control atherogenesis.

IL-13 is a potent stimulator of alternative monocyte/macrophage activation. During alternative activation, the expression of several proteins is induced including 15-lipoxygenase (15-LO), a lipid-peroxidating enzyme and the scavenger receptor CD36. We previously reported that $\alpha_M\beta_2$ integrin activation or clustering suppresses the expression of both 15-LO and CD36. In this study we focused on exploring the molecular mechanisms that down-regulate CD36 expression and CD36-mediated foam cell formation in IL-13-stimulated monocytes/macrophages after $\alpha_M\beta_2$ activation. Our studies reveal that $\alpha_M\beta_2$ integrin activation inhibits the IL-13 activation of several critical pathways that are required for macrophage alternative activation; namely, blocking Jak2 and Tyk2 phosphorylation, which bind to the cytoplasmic tails of the IL-4R and IL-13R complex. This leads to the inhibition of tyrosine phosphorylation of Stats (Stat1, Stat3, and Stat6) and prevents the formation of a signaling complex (containing p38MAPK, PKC, and Stat3) that are critical for the expression of both 15-LO and CD36. Jak2-mediated Hck activation is also inhibited, thereby preventing Stats serine phosphorylation, which is essential for downstream Stat-dependent gene transcription. Moreover, inhibition of Jak2, Tyk2, or their downstream target 15-LO with antisense oligonucleotides profoundly inhibits IL-13-induced CD36 expression and CD36-dependent foam cell formation, whereas the Hydroperoxyoctadecadienoic acid (HPODE), a 15-LO product and peroxisome proliferator-activated receptor-γ ligand, completely restores CD36 expression in monocytes treated with 15-LO antisense. $\alpha_M\beta_2$ integrin activation controls CD36 expression and foam cell formation in alternatively activated monocyte/macrophages by blocking Tyk2/Jak2 phosphorylation via a 15-LO-dependent pathway. The discovery of this mechanism helps our understanding of the potential role of alternatively activated macrophages in atherogenesis and highlights the impact of integrin $\alpha_M\beta_2$ on this process.

Monocytes/macrophages play a key role in innate and adaptive immunity and are crucial mediators of the inflammatory response (1). They have been shown to display phenotypic plasticity depending on their environment and cytokine exposure. During inflammation macrophages can be activated by the classical activation pathway mediated by IFNγ producing macrophages with the M1 phenotype (2) or by the alternative activation pathway induced by Th2 cytokines IL-4 and IL-13, producing M2 macrophages (3, 4). The relative roles of the various macrophage phenotypes in different inflammatory diseases have not been definitively elucidated.

Atherosclerosis is a chronic inflammatory disease in which macrophages play a central role. In response to inflammatory stimulation, monocytes migrate to the vessel intima, differentiate to macrophages, and accumulate lipids, forming foam cells, the hallmark of atherosclerosis. Current observations demonstrate that early lesions in apoE−/− mice are formed mostly by M2 macrophages, which are later transformed to the M1 phenotype due to proinflammatory cytokines (5). In agreement with this, another study revealed that in vitro treatment of M2 macrophages with oxLDL initiates the development of a strong proinflammatory response that shifts the M2 phenotype toward M1 (6). These studies show the potential pro-atherogenic role of M2 macrophages. Our study focuses on the mechanistic pathways mediated by alternative activation of monocytes/macrophages, which regulate the expression of a critical component of foam cell formation, the scavenger receptor CD36.

CD36 is a class B scavenger receptor expressed in a variety of cells including monocytes and macrophages. Macrophage CD36 has been implicated in atherogenesis by contributing to foam cell formation in the atherosclerotic blood vessel intima (7–9). The augmentation of CD36 expression has been shown...
after macrophage activation with IL-4 (10) or IL-13 (11) and may account for the potential pro-atherogenic functions of M2 macrophages.

We recently found that the stimulation of another essential macrophage pathway interferes with IL-13-mediated expression of CD36: the activation of integrin \( \alpha_M \beta_2 \) (12). Integrin \( \alpha_M \beta_2 \) (CD11b/CD18, MAC-1) is a cell surface receptor that is involved in adhesion/migration of monocytes and serves as a dynamic link between extracellular matrix and cytoskeleton (13). Integrin activation, which regulates the adhesion/migration capability of cells, is a critical step during the recruitment of monocytes to the inflamed intima. In parallel, integrin-mediated signaling regulates many important cell responses during macrophage adhesion and migration (14). The purpose of our current work was to analyze the detailed mechanism of \( \alpha_M \beta_2 \)-mediated regulation of CD36 expression during the alternative activation of macrophages and to evaluate the possible effect of this regulation on foam cell formation.

In our previous work we found that \( \alpha_M \beta_2 \) activation also suppresses the induction of 15-lipoxygenase (15-LO)\(^3\) (12). 15-LO is a lipid-peroxidizing enzyme that catalyzes the formation of 15(S) HPETE (Hydroperoxyeicosatetraenoic acid) from arachidonic acid and 13(S) HPODE from linoleic acid (15, 16). These products are potent mediators of inflammation (17) and are involved in the development of atherosclerosis (18–20). Previous studies demonstrated the potential link between 12/15-LO and CD36 expression in IL-4-stimulated mouse peritoneal macrophages (21). We hypothesized that \( \alpha_M \beta_2 \)-mediated inhibition of 15-LO and CD36 expression in human monocytes are related events during the activation of a single linear pathway rather than representing independent parallel pathways. We, therefore, tested the hypothesis that \( \alpha_M \beta_2 \)-mediated inhibition of CD36 expression and related foam cell formation are regulated via signaling pathways, which are critical for 15-LO induction.

We have found that inhibition of CD36 expression due to integrin \( \alpha_M \beta_2 \) activation is mediated by blocking the activation of the IL-13 receptor-associated Jak kinases (Jak2 and Tyk2), disruption of signaling complex Stat3/PKC\( \beta \)/p38MAPK, and inhibition of tyrosine and serine phosphorylation of Stat transcription factors. We further demonstrate that \( \alpha_M \beta_2 \) integrin activation reduces CD36-dependent foam cell formation via 15-LO-dependent mechanism. Thus, our findings reveal the molecular mechanism of \( \alpha_M \beta_2 \)-mediated inhibition of foam cell formation and demonstrate the influence of 15-LO in controlling CD36 expression and function in alternatively activated human monocytes/macrophages by IL-13.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Recombinant human IL-13 was purchased from Invitrogen. The rabbit reticulocyte 15-LO antibody, cross-reacting with human 15-LO, was raised in sheep and was obtained as a gift from Dr. Joseph Cornicelli (Parke-Davis). Anti-phosphotyrosine-Stat (Tyr(P)-701-Stat1, Tyr(P)-705-Stat3, and Tyr(P)-641-Stat6), anti-phosphoserine-Stat3 (Ser(P)-727-Stat3), and p38MAPK antibodies were purchased from Cell Signaling Technology (Beverly, MA) and diluted 1:1000 according to the manufacturer’s protocol. Stat3 monoclonal antibody was from BD Transduction Laboratories. The other primary antibodies used in this study were mouse anti-human \( \beta \)-Tyr (PY99), anti-Jak1, anti-Jak2, anti-Tyk2, anti-Hck, anti-phospho-(Tyr-411)-Hck (affinity purified goat polyclonal antibody raised against a short amino acid sequence containing phosphorylated Tyr-411 of Hck of human origin), rabbit anti-human PKC\( \delta \) (C-20), and \( \beta \)-tubulin from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-CD36 polyclonal antibody was purchased from Cayman Chemical (Ann Arbor, MI). FITC-conjugated anti-CD36 mAb was obtained from BD Biosciences. FITC-conjugated anti-CD14 mAb (Clone 61D3) was from eBioscience (San Diego, CA). The mAb 44a directed against the \( \alpha_M \beta_2 \) integrin subunit was purified from the conditioned media of the hybridoma cell line obtained from American Type Culture Collection (Manassas, VA) using protein A-agarose (GE Healthcare). Activating anti-\( \beta \)-2 antibody (clone MEM48) was purchased from Millipore (Billerica, MA). Rosiglitazone, GW9662, PD146176, 13(S) HPODE and 13(S) HODE were from Cayman Chemical (Ann Arbor, MI). The inhibitors were dissolved in dimethyl sulfoxide and stored either at 4 °C or at −20 °C as concentrated stock solutions according to the manufacturer’s instructions.

**Isolation of Human Monocytes**—Human peripheral blood monocytes were isolated and purified from whole blood as described previously (22). Briefly, PBS-diluted blood was layered over a Ficoll-Paque density solution and centrifuged. The mononuclear cell layer was collected, and contaminating platelets were removed by centrifugation through bovine calf serum (BCS) after overlaying the serum with the mononuclear cells. Monocytes were isolated from the platelet-free mononuclear cells by adherence to flasks precoated with BCS and containing DMEM and 10% BCS. The flasks were incubated for 2 h at 37 °C in 10% CO\(_2\). Non-adherent cells were removed by washing the flasks with DMEM. Adherent cells were detached with PBS containing 5 mM EDTA. The monocytes were collected, washed 3 times, and incubated at 37 °C in 10% CO\(_2\), for at least 2 h before use in experiments. The purity of the monocytes was 90%, which was confirmed for each experiment by FACS analysis with anti-CD14 mAb. In some experiments primary human monocytes were incubated at 37 °C in 10% CO\(_2\) in the presence of DMEM and 10% BCS for another 5 days to be differentiated into monocyte-derived macrophages. We refer to these cells as “macrophages” and activated them with IL-13 at this stage. These studies complied with all relevant federal guidelines and institutional policies regarding the use of human subjects.

**Analysis of CD36 Expression by FACS**—FACS analyses were performed to assess the expression of CD36 on the surface of monocytes. Monocytes were harvested and washed in Hanks’ buffer two times. The cell pellet was resuspended in Hanks’ buffer, and then 2 × 10\(^6\) cells were preincubated with 4% normal goat serum for 30 min at 4 °C. Cells were pelleted by centrifugation, resuspended in Hanks’ buffer, and incubated with specific FITC-conjugated mouse anti-human CD36 mAb (BD Biosciences) for 30 min at 4 °C according to a protocol supplied...
Regulation of CD36 Expression by αMβ2 Integrin

by the manufacturer. Finally, the cells were washed, resuspended in PBS, and then analyzed using a FACScan™ (BD Biosciences). For each experiment an appropriate nonspecific isotype antibody was used as a negative control.

Immunoprecipitation and Immunoblotting—Peripheral blood monocytes were treated with IL-13 (2 nM) for different time intervals as indicated, and postnuclear and total cell extracts were prepared as described previously (23). After determining the protein concentration using the Bio-Rad protein assay reagent, lysate proteins (50 μg/well) were resolved by 8% SDS-PAGE, transferred to a PVDF membrane, blocked with 5% BSA in PBS with 0.1% Tween 20, and subjected to immunoblotting with CD36, phospho-Stats or phospho-Hck antibodies overnight. The hybridization signal was detected using SuperSignal West Pico chemiluminescent substrate (Pierce). 15-LO protein was detected on Western blots following a previously described protocol (22). For immunoprecipitation, the lysates were incubated with either Jak2, Tyk2, or PKCδ antibodies and precipitated with prewashed Protein A-Sepharose beads (Sigma) at 4 °C overnight. The precipitates were boiled for 5 min in SDS sample buffer and subjected to immunoblotting as described above. In several experiments immunoblots were stripped and reprobed to assess equal loading according to our previously published protocol (23).

Quantitative Real-time PCR—Monocytes (5 × 10⁶ in 2 ml 10% BCS/DMEM) were plated in six-well culture plates. After plating overnight for peripheral blood monocytes, cells were treated with recombinant IL-13 (2 nm) for 24 h. In some experiments, monocytes were pretreated with different pharmacological inhibitors for 30 min followed by IL-13 treatment for 24 h. After treatment monocytes were collected and washed with PBS. Total cellular RNA was extracted using the RNeasy mini kit from Qiagen (Valencia, CA), and real-time quantitative RT-PCR was performed according to established protocols (24). The sequences of the 15-LO primers were: forward (5’-GCTGGAAAGGATCGTATGACT-3’) and reverse (5’-TGGCTACAGAGATAGCGTGG-3’). The sequences of the CD36 primers were: forward (5’-CAGAGGCTGACACTCAGACAG-3’) and reverse (5’-AGGGTACCGAACAATCACCCG-3’). GAPDH was used as an internal control with the primer sequences of: forward (5’-CACAACCTGCGATTTCCCACCG-3’) and reverse (5’-TGCTCATGATGGTCCACCG-3’).

Monocyte Stimulation with β2 Integrin Activation Antibody or Clustering via αM Integrin—Monocytes (5 × 10⁶/2 ml) were incubated overnight, and then the activation antibody to β2 integrin (clone MEM48, 10 μg/ml) was added to the cells and incubated for 3 min before IL-13 stimulation. Mouse IgG (10 μg/ml) was added to a separate well as a control. For integrin clustering monocytes were treated with anti-αM antibody (clone 44a, 5 μg/ml) for 20 min. After incubation goat-antimouse antibody was added (2 μg/ml) for an additional 5 min followed by treatment with IL-13. For the clustering control only anti-αM antibody (5 μg/ml) was added to the monocytes before IL-13 treatment.

Treatment of Monocytes with Jak1, Jak2, and Tyk2 Antisense Oligodeoxyribonucleotides (ODNs)—The antisense ODN sequences for human Jak1, Jak2, and Tyk2 were selected based on our previously published studies (22). Control ODN for Jak2 and Tyk2 consisted of complementary sense ODN. The sense ODN sequence for Jak1 was predicted to possibly serve as an antisense for human γ-adaptin mRNA. Hence we chose a scrambled ODN as a control for the Jak1 antisense. All ODNs were end-modified (phosphorothioated, three bases at the 5’ and 3’ ends) to limit DNA degradation, and all were HPLC-purified before use (Invitrogen).

The sequences of the ODNs are as follows: Jak2 antisense, 5’-TCTTAACCTCGTTCTCGTTC-3’; Jak2 sense, 5’-GAAC-GAGAACAGAGTTAAGA-3’; Tyk2 antisense, 5’-CCAACTTTATGTGCAAAGTTG-3’; Tyk2 sense, 5’-CACATGGCACAATAAGTTG-3’. The antisense ODNs were annealed with the sense ODNs along with the sense and scrambled ODN controls for 48 h with one re-feed at 24 h before the addition of IL-13.

Treatment of Cells with 15-LO Antisense ODN—15-LO sense and sense ODNs were obtained from Invitrogen and handled according to the manufacturer’s instructions. The 15-LO antisense ODN sequence was 5’-CCGTGACAGATGCAGTGTC-3’. A complementary 15-LO sense ODN sequence was used as a control. All ODNs were end-modified (phosphorothioated, three bases of 5’ and 3’) to limit DNA degradation, and all were HPLC-purified before use. 15-LO antisense or sense ODNs were boiled for 2–3 min and then cooled at room temp before being added to cells. Human monocytes (5 × 10⁶ in 2 ml 10% BCS/DMEM) were fed either with 15-LO sense or antisense ODNs (5 μM). To check the effect of 15-LO antisense ODN on 15-LO protein expression, IL-13 (2 mM) was added to the appropriate samples 2 h after the antisense treatment. Cells were allowed to incubate at 37 °C and were re-fed with 15-LO sense or antisense ODNs (5 μM) after 24 h. Cells were then harvested and assayed for silencing 48 h after 15-LO antisense treatment. For real-time PCR experiments with the 15-LO antisense ODNs, cells were treated with 15-LO sense or antisense ODNs (5 μM) for a total period of 48 h with one re-feed after 24 h before the stimulation with IL-13 for 24 h.

Transfection of Stat1 and Stat3 Decoy and Mismatched Oligodeoxyribonucleotides into Monocytes—The phosphorothioated ODNs used for Stat1 and Stat3 decoys were purchased from Invitrogen. These decoys were used in previous studies and shown to provide specific inhibition of Stat1 and Stat3 activities (21). The sequences for the decoys were 5’-ATA TTC CTG TAA GTG-3’ and 3’-TAT AAG GAC ATT CAC-5’ for Stat1, 5’-ATA TTG GAG TAA GTG-3’ and 3’-TAT AAG CTC ATG CAC-5’ for the Stat3 decoy, and 5’-GAT CCT TCT GGG AAT TCC TAG ATC-3’ and 3’-CTA GGA AGA CCC TTA AGG ATC TAG-5’ for the Stat3 decoy. The single-stranded ODNs were annealed by incubation at 65 °C for 10 min and then slowly cooled to room temperature for 2 h. Double-stranded decoys were transfected into monocytes at a final concentration of 2 μM by using Superfect (Qiagen) following the manufacturer’s instructions. After 24 h of transfection cells were treated with IL-13 for 24 h to assess CD36 mRNA expression.
Transfection of Stat6 Decoy and Scrambled Decoy ODNs into Monocytes—Double-stranded decoy ODNs containing the conserved promoter binding site of the Stat6 transcription factor and a scrambled decoy ODN sequence were prepared from complementary single-stranded phosphorothioate-modified oligonucleotides (ordered from Invitrogen) by melting at 90 °C for 3 h during which time the temperature was reduced from 90 °C to 25 °C. Human monocytes were plated in six-well culture plates overnight. Cells were then transfected with decoy ODNs using Superfect Transfection Reagent (Qiagen) according to the manufacturer’s instructions for 24 h. Monocytes were then incubated in the absence or presence of IL-13 for another 24 h for CD36 mRNA quantification. The single-stranded sequences of the decoy ODNs were as follows: Stat6 decoy ODN (5'-GAT CAA GAC CTT TTC CCA AGA AAT CTA T-3' and 3'-CAT GTT CTG GAA AAG GGT TCT TTA GAT A-5'); scrambled decoy ODN (5'-CGA AAA TTC GTC AAA TCA CTA GCT TAC C-3' and 3'-GCT TTT AAG CAA TTT AGT GAT CGA ATG G-5').

Foam Cell Formation Assay—The LDL, modified by reactive nitrogen species generated by the leukocyte myeloperoxidase system (NO2 oxLDL) was kindly provided by Dr. David Kennedy (from Dr. Roy Silverstein laboratory, Lerner Research Institute, Cleveland, OH). Fluorophor-labeled NO2 oxLDL was generated using 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) according to the manufacturer’s instructions (Molecular Probes, Eugene, OR). Primary human monocytes were plated on coverslips (2.5 × 10⁶/ml) and differentiated 5 days in DMEM medium supplemented with 10% lipoprotein-deficient serum. 1 h before the DiLDL treatment, the medium was exchanged to serum-free DMEM medium. Cells were incubated for 30 min in the presence of 5 μg/ml DiI-oxLDL. After incubation, coverslips were washed 3 times with cold PBS, fixed with 10% formaldehyde, and mounted on slides in DAPI solution. DiI-oxLDL was detected by fluorescence microscope. Five randomly selected fields from each slide were analyzed. The intensity of the DiI fluorophor was quantified using computer-assisted image analysis software Image-Pro Plus (Media Cybernetics, Bethesda, MD).

Statistical Analysis—Statistical analyses were performed using Student’s t test for the FACS analysis and foam cell formation experiments and Student’s paired t test for the real-time PCR experiments. A value of p < 0.05 was considered significant.

RESULTS

αMβ2 Integrin Activation or Clustering Attenuates Stat3 Tyrosine Phosphorylation, PKCδ-Stat3 Association, and PKCδ-mediated Stat3 Serine Phosphorylation in IL-13-induced Monocytes—The up-regulation of CD36 and 15-LO expression after IL-13/IL-4 treatment has been shown before (11, 22, 25, 26). In our recently published work we found that IL-13-induced expression of CD36 and 15-LO is inhibited by the activation of integrin αMβ2 (12). In this report we explored the molecular mechanism of αMβ2-mediated inhibition of CD36 expression. To expand our previous results we investigated the elevated expression of CD36 in alternatively activated monocytes/macrophages by FACS to analyze the surface expression (supplemental Fig. S1A) and by Western blotting to detect total CD36 protein (both cell surface and intracellular protein) expression in whole cell lysates (supplemental Fig. S1B). Although we observed a considerable increase in IL-13-induced CD36 expression by Western blotting at day 4 (supplemental Fig. S1B), the level of CD36 on the cell surface was only slightly increased (supplemental Fig. S1A). In contrast, after 5 days of IL-13 incubation, we detected significant changes in both total CD36 protein level and surface expression of CD36. These results demonstrate that after day 4, although total CD36 protein expression was significantly enhanced, the expression of CD36 on the surface was somewhat delayed.

We next examined the time-dependent effect of αMβ2 integrin activation on CD36 protein expression after incubation of monocytes with IL-13. Therefore, for these studies we used the anti-integrin β2 activation antibody (clone MEM48). Our data show that αMβ2 integrin activation inhibits IL-13-induced CD36 protein expression almost completely in monocytes/macrophages after 4 and 5 days of incubation with IL-13 (supplemental Fig. S1B).

We have previously published that IL-13-mediated Stat3 tyrosine phosphorylation is required for the formation of a molecular signaling complex (signalosome) containing p38MAPK (data not shown), PKCδ, and tyrosine- phosphorylated Stat3 (27). The signalosome was shown to regulate Stat3 Ser-727 phosphorylation and Stat3-dependent transcription of 15-LO (27). Based on these results we tested whether αMβ2 integrin activation or clustering affected IL-13-stimulated Stat3 tyrosine phosphorylation, Stat3 association with PKCδ, and Stat3 Ser-727 phosphorylation to determine whether integrin activation affected these aspects of IL-13 signaling. Our results indicate that IL-13-induced Stat3 Tyr-705 phosphorylation is substantially inhibited by both αMβ2 integrin activation and clustering, whereas the corresponding controls are without effect (Fig. 1A). As a consequence, we investigated the effect of αMβ2 integrin activation and clustering through αM integrin, the molecular association between Stat3 and PKCδ was greatly diminished (Fig. 1B). In similar conditions, the activation control (mouse IgG at the same concentration) and clustering control (only primary anti-αM antibody) caused no inhibition of the IL-13-dependent association of Stat3 with PKCδ (Fig. 1B). We next examined the effect of αMβ2 integrin activation and clustering on IL-13-induced Stat3 Ser-727 phosphorylation. The results demonstrate that when the IL-13-stimulated molecular complex formation (between Stat3 and PKCδ is suppressed by either αMβ2 integrin activation or clustering, PKCδ-mediated Stat3 Ser-727 phosphorylation was also inhibited (Fig. 1C). Hence our results suggest that the down-regulation of IL-13-stimulated 15-LO expression by αMβ2 integrin activation or clustering is associated with interference of IL-13-induced signaling events starting from the inhibition of Stat3 tyrosine (Tyr-705) phosphorylation and abolition of the molecular complex formation between PKCδ and Stat3 followed by the abrogation of PKCδ-mediated Stat3 Ser-727 phosphorylation.
**Regulation of CD36 Expression by αMβ2 Integrin**

**FIGURE 1. Activation of Stat 3 is inhibited by αMβ2 integrin activation and clustering in IL-13-stimulated human monocytes.** Monocytes were incubated overnight, and then the activation antibody to β2 integrin was added to the cells before IL-13 stimulation for either 15 min (A and B) or 1 h (C). For integrin clustering, monocytes were incubated with anti-αM antibody and clustered with goat-anti-mouse antibody followed by treatment with IL-13 for either 15 min (A and B) or 1 h (C). 50 μg of cell lysate was subjected to Western blot analysis using anti-phospho-Tyr-701 Stat1 (upper panel of A) and anti-phospho-Ser-727 Stat3 (upper panel of C) antibodies. The membranes were stripped and reprobed with anti-β-tubulin antibody to assess equal loading (lower panels of A and C). In panel B, whole cell lysates were immunoprecipitated with PKCβ antibody. The immunoprecipitates (IP) were then resolved by 8% SDS-PAGE for immunoblotting with Stat3 monoclonal antibody (upper panel of B). The blot was stripped and reprobed with PKCβ antibody (lower panel of B) to confirm equal immunoprecipitation.

**FIGURE 2. Stat1 and Stat6 tyrosine phosphorylation is inhibited by αMβ2 integrin activation and clustering in IL-13-induced primary human monocytes.** Freshly isolated human peripheral blood monocytes (5 × 10^6/2 ml of 10% BCS/DMEM) were incubated overnight, and then the activation antibodies to β2 integrin (MEM48) (10 μg/ml) was added to the cells and incubated for 3 min before IL-13 stimulation (2 nM) for either 15 min (A and B). Mouse IgG (10 μg/ml) was added to the separate well as control. For integrin clustering, monocytes were treated with anti-αM antibody (5 μg/ml) for 20 min. After incubation goat-anti-mouse antibody was added (2 μg/ml) for an additional 5 min followed by treatment with IL-13 for either 15 min (A and B). For the clustering control only anti-αM antibody (5 μg/ml) was added to the monocytes before IL-13 treatment. 50 μg of cell lysate was subjected to Western blot analysis using anti-phospho-Tyr-701 Stat1 (upper panel of A) and anti-phospho-Tyr-641 Stat6 (upper panel of B) antibodies. The membranes were stripped and reprobed with anti-β-tubulin antibody to assess equal loading (lower panels of A and B).

αMβ2 Integrin Activation or Clustering Inhibits Tyrosine Phosphorylation of Both Stat1 and Stat6—As Stat1 and Stat6 also regulate IL-13-dependent transcription of 15-LO in primary human monocytes, we next investigated the effect of αMβ2 integrin activation or clustering on Stat1 and Stat6 activation in response to IL-13 stimulation. Our results show that both Stat1 and Stat6 tyrosine phosphorylation (Tyr-701 and Tyr-641 residues, respectively) are substantially blocked by either αMβ2 integrin activation or clustering after IL-13 stimulation (Fig. 2, A and B). Similar to our previous observations, the activation and clustering controls show no inhibition of IL-13-dependent tyrosine phosphorylation of Stat1 and Stat6 (Fig. 2, A and B).

αMβ2 Integrin Activation Blocks Key Upstream Signaling Pathways; Jak2 and Tyk2 Phosphorylation—As tyrosine phosphorylation of Stats (Stat1, Stat3, and Stat6) is attenuated by αMβ2 integrin activation, we further examined whether the upstream signaling pathways of Stat activation (tyrosine phosphorylation), like the activation of IL-13-receptor-associated Jak kinases, are also blocked. Our results demonstrate that when IL-13-induced tyrosine phosphorylation of Stats is inhibited during αMβ2 integrin activation, the activation/tyrosine phosphorylation of upstream Jak kinases (Tyk2 and Jak2) are substantially down-regulated (Fig. 3, A and B). In similar conditions, the activation control (mouse IgG at the same concentration) shows no inhibition on IL-13-dependent tyrosine phosphorylation of both Tyk2 and Jak2 (Fig. 3, A and B). In parallel experiments we sought but did not find direct association between IL-4/αβ-IL-13Rα1 and αMβ2 after integrin αMβ2 activation nor any difference in the IL-4/αβ-IL-13Rα1 expression before and after activation (data are not shown).

αMβ2 Integrin Activation Blocks Jak2-mediated Hck Phosphorylation—Hck is a key upstream signaling pathway in IL-13-activated monocytes/macrophages. Previously we dem-
**Regulation of CD36 Expression by α₉β₂ Integrin**

Our results demonstrated that α₉β₂ integrin activation has no effect on Hck expression level in alternatively activated monocytes/macrophages (Fig. 3C, bottom panel) but provided strong evidence that α₉β₂ integrin activation significantly blocks Hck activation (Tyr-411 phosphorylation), whereas the activation control had no effect (Fig. 3C, upper panel). These results further show that Hck-mediated signaling, which is important for both 15-LO and CD36 expression, is also modulated by β₂ integrin activation in alternatively activated monocytes/macrophages.

**Jaks (Jak2 and Tyk2) and Stats (Stat1, Stat3, and Stat6) Are Required for CD36 Expression in Alternatively Activated Monocytes by IL-13**—Because α₉β₂ integrin activation and clustering blocked Jak2 and Tyk2 phosphorylation and Stat3 dual phosphorylation (both Tyr-705 and Ser-727) as well as Stat1 and Stat6 tyrosine phosphorylation, we hypothesized that these Jaks and Stats may also regulate CD36 expression. To address this question, the effect of Jak1-, Tyk2-, and Jak2-specific antisense and scrambled/sense ODNs was also monitored on CD36 expression in response to IL-13 stimulation. Our results showed that Tyk2 antisense ODNs significantly attenuated CD36 protein expression after IL-13 activation (Fig. 4A, upper panel) as compared with sense ODN controls. In contrast, Jak1 antisense ODN caused no inhibition of IL-13-activated CD36 protein expression (Fig. 4A, upper panel). Antisense ODN inhibition of Jak1 and Tyk2 protein expression levels were also verified by reprobing the same blot with antibodies against Jak1 and Tyk2 kinases (Fig. 4A, last two panels). By performing similar experiments as above, we further showed that substantial inhibition of Jak2 expression in monocytes treated with antisense ODN to Jak2 (Fig. 4B, bottom panel) was also associated with substantial down-regulation of CD36 protein expression after IL-13 activation (Fig. 4B, upper panel). These results thus clearly demonstrate that both Jak2 and Tyk2 are required for the IL-13-mediated up-regulation of CD36 expression in monocytes/macrophages. To further show the requirement of Stats in regulating CD36 expression, we used Stat1, Stat3, and Stat6 decoy ODNs and their corresponding mismatched/scrambled ODNs as controls to transfect monocytes. In our previously published studies we demonstrated that transfection of monocytes with either the Stat1 or Stat3 decoy ODNs markedly attenuated Stat1 and Stat3 DNA binding activities induced by IL-13, respectively, compared with that of untransfected cells or cells transfected with mismatched ODNs (24). Previous studies by Wang et al. (29) also showed that Stat6 decoy ODN specifically inhibited IL-4-induced Stat6 DNA binding activity.

The cells were stimulated with IL-13, and the effect of transfection of these decoy ODNs on CD36 mRNA expression was assessed. Transfection of monocytes with either Stat1 or Stat3 or Stat6 decoy ODNs markedly reduced IL-13-induced CD36 mRNA expression (Fig. 5, A–C) as compared with untransfected monocytes (IL-13-treated control) or transfection controls (*, p < 0.001; **, p < 0.003). Transfection of cells with either Stat1 or Stat3 mismatched ODNs or Stat6 scrambled ODNs (controls) had no significant effect on IL-13-stimulated CD36 mRNA expression (Fig. 5, A–C). These results thus provide evidence that, similar to the regulation of 15-LO expression, Jaks (Jak2 and Tyk2) and Stats (Stat1, Stat3, and Stat6) are
all critical regulators of CD36 expression in alternatively activated monocytes.

**IL-13-dependent CD36 Expression on Human Monocytes/Macrophages Requires 15-LO Expression/Activity**—The potential link between 12/15-LO induction and CD36 expression has been shown before in mouse peritoneal macrophages stimulated with IL-4 (30). Based on our observations that both CD36 and 15-LO are inhibited after activation in human primary monocytes (12), we next examined whether IL-13 induction of 15-LO expression is upstream of CD36 gene expression in our system. To test this hypothesis, we first determined the effect of 15-LO antisense ODN on 15-LO expression at both the mRNA and protein levels. Cells were treated with the 15-LO-specific antisense or control sense ODN and then exposed to IL-13 (Fig. 6, A and B). Total cellular RNA or proteins were extracted for real-time RT-PCR or Western blot analysis. Our results indicate that the 15-LO specific antisense ODN significantly inhibited the IL-13-mediated induction of 15-LO protein expression (Fig. 6A) and 15-LO mRNA expression (Fig. 6B) (*, p < 0.05), whereas the 15-LO sense ODN had no effect (Fig. 6A and B). Because CD36 expression has previously been shown to be regulated by PPAR and 15-LO generates the PPAR ligand HPODE, we next explored the possibility that
Induction of CD36 mRNA expression by IL-13 was also significantly blocked (*, p < 0.05) by pretreatment with a selective inhibitor of 15-LO activity PD146176 in a dose-dependent manner (Fig. 6C). In contrast, the addition of the 15-LO product 13(S) HPODE, which is also a PPARγ ligand, profoundly up-regulated the expression of CD36 mRNA in monocytes (Fig. 6C). Similar to CD36 mRNA, expression of CD36 protein was also evaluated after treatment with 15-LO antisense or sense ODNs followed by incubation with IL-13 for 5 days. The addition of 15-LO antisense substantially inhibited the IL-13 induction of CD36 expression on the surface of human monocytes, whereas the 15-LO sense had no effect (Fig. 6D). To confirm our FACS data we also examined the effect of 15-LO antisense on CD36 protein expression by performing an immunoblot analysis. Our results in Fig. 6E show that IL-13 profoundly up-regulated CD36 protein expression. Treatment with the 15-LO-specific antisense ODN significantly inhibited the IL-13-induced CD36 protein expression (Fig. 6E). Conversely, 15-LO sense ODN had no inhibitory effect on IL-13-stimulated protein expression of CD36 (Fig. 6E). These results provide evidence that the suppression of IL-13-stimulated CD36 expression by αMβ2 integrin activation or clustering is directly associated with the down-regulation of 15-LO expression/activity in alternatively activated monocytes.

15-LO Expression/Activity Is Critical for CD36-related Foam Cell Formation in IL-13-stimulated Human Monocytes/Macrophages—Previously we found that αMβ2 activation inhibited CD36-related foam cell formation (12). To demonstrate the critical role of 15-LO in this process, we tested the effect of 15-LO antisense on oxidized lipid uptake. Therefore, monocytes were pretreated with 15-LO sense and antisense ODNs followed by IL-13 stimulation for 5 days. We exposed monocyte-differentiated macrophages to a fluorophore (DiI)-tagged form of NO2-oxidized LDL (DiI-NO2 LDL) and measured DiI-NO2 LDL internalization by fluorescence microscopy (Fig. 7A). Our data show that uptake of NO2-oxidized LDL, a preferential ligand for CD36 (31), was significantly increased in macrophages differentiated in the presence of IL-13, and this internalization was dramatically attenuated by 15-LO antisense ODN. In contrast, 15-LO sense ODN showed no inhibition of IL-13-induced foam cell formation (Fig. 7B). The inhibitory effect of 15-LO antisense ODN treatment on IL-13-stimulated CD36 expression and foam cell formation was totally rescued by the addition of the 15-LO product 13(S)-HPODE. These results demonstrate that 15-LO is a critical component of IL-13-mediated foam cell formation. Notably, 13(S)-HPODE as well as 13(S)-HODE can stimulate foam cell formation (Fig. 7, A and B) and CD36 expression (Fig. 7C) on monocytes in the absence of IL-13 providing additional confirmation of our conclusion.

As 13-HPODE is a well known ligand for PPARγ, we next investigated the role of PPARγ in mediating IL-13-induced CD36 expression and CD36-dependent foam cell formation in our experimental model. To confirm that PPARγ is required for CD36-related foam cell formation, we inhibited PPARγ function by GW9662 (a PPARγ antagonist) (supplemental Fig. S2B). The involvement of PPARγ in IL-13-dependent CD36 expression was also confirmed by real-time PCR (supplemental Fig.
Regulation of CD36 Expression by αMβ2 Integrin

**FIGURE 7.** 15-LO antisense inhibits IL-13 mediated foam cell formation, whereas 13-HPODE rescues oxidized lipid uptake. Human monocytes were treated with 15-LO antisense (AS) or 15-LO sense (S) ODNs (5 μM) and stimulated with IL-13. In some groups 13(S)-HPODE (20 μM) and 13(S)-HODE (20 μM) were added either directly (for 5 days) or after 24 h of IL-13 incubation (HPODE addition in AS-treated group) as described in the panels. **A**, after 5 days lipid uptake was evaluated using Dil-oxLDL (magnification ×400). Data are from a representative experiment of 3–6 (for different samples) that was performed. **B**, five randomly selected fields from each slide were analyzed, and Dil intensity was quantified using the computer-assisted image analysis software Image-Pro Plus. Data were normalized based on the total number of cells in a particular field and plotted as the mean ± S.D. (*, p < 0.05; **, p < 0.01). **C**, cells from the same experiment were lysed, and 30 μg of the post nuclear extracts (from each sample group) were separated by 8% SDS-PAGE and immunoblotted with anti-CD36 antibody (upper panel of C). The same blot was stripped and reprobed with β-tubulin antibody to assess equal loading (lower panel of C). The legend for panels B and C is common.

S24), FACS analysis (Fig. 6D), and Western blotting (Fig. 6E) using the PPARγ antagonist GW9662 (in IL-13-treated monocytes) and the agonist rosiglitazone (in non-treated monocytes). In contrast, our recent results showed that treatment of monocytes/macrophages with GW9662 caused no inhibition of IL-13-induced 15-LO expression, suggesting that IL-13-mediated induction of 15-LO is PPARγ-independent (12). Thus, 15-LO is upstream of PPARγ, whereas CD36 is downstream, and 15-LO expression/activity is required for regulation of CD36 expression and CD36-mediated foam cell formation in IL-13-stimulated monocytes.

**DISCUSSION**

CD36 is a multiligand scavenger receptor involved in several physiological functions; however, during atherogenesis scavenger abilities of CD36 can lead to pathophysiological foam cell formation. In this study we show that IL-13-driven alternative activation of macrophages leads to enhanced expression of CD36, and this up-regulation is suppressed by αMβ2 integrin activation. Integrin activation is an essential step during monocyte migration from the blood stream through the vessel wall to the site of inflammation or during the macrophage trapping in the inflamed subendothelial extracellular matrix. Thus, αMβ2 integrin-mediated regulation of CD36 expression may have broad implications for the physiology and pathophysiology associated with the inflammatory states, particularly atherosclerosis. Recently the contribution of integrin αMγ to the development of atherosclerosis was examined using αM/ApoE double-deficient mice fed a Western diet for 16 weeks (32). The result showed that αMγ deficiency in female mice increases atherosclerotic lesions. These data are supported by our recent studies (12) where we showed that the uptake of Dil-labeled oxLDL was increased in peritoneal macrophages isolated from αMγ-deficient mice. These experiments demonstrate the atheroprotective effect of αMγ using in vivo approaches and are consistent with the central hypothesis of our study.

Our data are consistent with the previously published observation that IL-13 treatment up-regulates CD36 expression on human monocytes (11). In addition, we show that this stimulation has a prolonged effect on CD36 expression that manifests in significant augmentation of CD36 protein levels at 5 days after IL-13 treatment. CD36 is not highly expressed on the surface of monocytes. In our studies CD36 protein expression occurs only after monocyte differentiation to the macrophages. We detected remarkable changes in the cell shape and spreading after 4–5 days that indicates macrophage differentiation. Therefore, although the signal for the change in CD36 expression was initiated after IL-13 stimulation the protein expression also required macrophage maturation.

It has previously been reported that the coordinate induction of PPARγ and 12/15-LO mediates IL-4-dependent transcription of the CD36 gene in mouse peritoneal macrophages (30). In our study using 15-LO antisense oligonucleotides, we demonstrate for the first time that CD36 gene expression is directly controlled by 15-LO expression/activity in IL-13-stimulated primary human monocytes/macrophages. Furthermore, we show that 15-LO product (and PPARγ ligand) 13-HPODE can rescue the inhibitory effect of 15-LO antisense oligonucleotides on both CD36 expression and CD36-related lipid deposition in monocytes/macrophages treated by IL-13. These data clearly demonstrate the consecutive contribution of 15-LO, PPARγ, and CD36 in IL-13-mediated oxidized lipid uptake in human monocyte-derived macrophages.

Importantly, we show a mechanistic link between endogenous 15-LO expression/activity and CD36 gene expression in the context of αMβ2 integrin activation. We demonstrate that αMβ2 integrin activation modulates key upstream signaling pathways like the IL-13 receptor-associated Jak kinases and interferes with the activation (tyrosine phosphorylation) of Stat transcription factors (Stat1, Stat3, and Stat6). αMβ2 integrin activation also attenuates Jak2-mediated Hck activation, which is also a crucial regulator of Stat serine phosphorylation. As Stat tyrosine and serine phosphorylation are both required for maximal efficiency of Stat-dependent gene transcription, our
results provide detail mechanistic evidence of $\alpha_M \beta_2$ integrin activation that blocks both 15-LO and CD36 gene expression in alternatively activated monocytes/macrophages. Moreover, our data show for the first time that the receptor-associated Jak kinases (Jak2 and Tyk2) are directly involved in regulating CD36 gene expression in IL-13-activated monocytes/macrophages. Altogether our results demonstrate the unique inhibitory role of $\alpha_M \beta_2$ integrin activation in Jak/Stat-mediated regulation of CD36 expression in alternatively activated monocytes/macrophages.

The recent observations demonstrate that M2 macrophages abound in the early atherosclerotic lesions (5) and generate proinflammatory mediators after engagement with oxLDL (6). These observations confirm the importance of $\alpha_M \beta_2$-mediated inhibition on the IL-13-generated signaling pathways during the development of atherosclerosis. Therefore, the mechanism for the inhibition of lipid deposition described in our study can be applied for macrophages activated toward M2 phenotype that utilize $\alpha_M \beta_2$ for the migration to the site of inflammation.

In summary, we report here the unique regulation of CD36 gene expression by IL-13-mediated activation of receptor-associated Jak kinases, downstream Stat transcription factors (Stat1, Stat3, and Stat6), and the involvement of a signaling complex (containing tyrosine phosphorylated Stat3 along with the Ser/Thr kinases PKC and p38MAPK (27)) regulating this process. $\alpha_M \beta_2$ integrin activation or clustering blocks Stat activation (Stat tyrosine and serine phosphorylation) by modulating two different signaling cascades, by blocking the activation of the receptor-associated Jak kinases (Jak2 and Tyk2), and by down-regulating the Jak2-mediated activation of Hck. One of the major effects of this is the suppression of the signaling complex formation and suppression of IL-13-stimulated 15-LO expression resulting in inhibition of PPARγ-mediated CD36 expression. These studies provide new mechanistic insights that explain how $\alpha_M \beta_2$ integrin activation inhibits CD36 expression during IL-13 activation of human monocytes.

Acknowledgments—We thank Meenakshi Shukla for providing freshly isolated monocytes for this study. We thank Dr. Richard Morton for providing lipoprotein-deficient serum. We are thankful to Drs. David Kennedy and Roy Silverstein for the NO2-oxidized LDL.

REFERENCES

1. Varin, A., and Gordon, S. (2009) Alternative activation of macrophages. Immune function and cellular biology. Immunochemistry 214, 630–641
2. Dalton, D. K., Pitts-Meek, S., Keshav, S., Figari, I. S., Bradley, A., and Stewart, T. A. (1993) Multiple defects of immune cell function in mice with disrupted interferon-γ genes. Science 259, 1739–1742
3. Gordon, S. (2003) Alternative activation of macrophages. Nat. Rev. Immunol. 3, 23–35
4. Gordon, S., and Martinez, F. O. (2010) Alternative activation of macrophages. Mechanism and functions. Immunity 32, 593–604
5. Khallou-Laschet, J., Varthaman, A., Fornas, G., Compan, C., Gaston, A. T., Clement, M., Dussiot, M., Levillain, O., Graff-Dubois, S., Nicoletti, A., and Caligiuri, G. (2010) Macrophage plasticity in experimental atherosclerosis. Plos one 5, e8852
6. van Tits, L. J., Stienstra, R., van Lent, P. L., Netea, M. G., Joosten, L. A., and

4 A. Bhattacharjee and M. K. Cathcart, unpublished observation.

Regulation of CD36 Expression by $\alpha_M \beta_2$ Integrin
Stalenhoef, A. F. (2011) Oxidized LDL enhances pro-inflammatory responses of alternatively activated M2 macrophages. A crucial role for Krüppel-like factor 2. Atherosclerosis 214, 345–349
7. Febbraio, M., Hajjar, D. P., and Silverstein, R. L. (2001) CD36. A class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. J. Clin. Invest. 108, 785–791
8. Rahaman, S. O., Lennon, D. J., Febbraio, M., Podrez, E. A., Hazen, S. L., and Silverstein, R. L. (2006) A CD36-dependent signaling cascade is necessary for macrophage foam cell formation. Cell Metab. 4, 211–221
9. Silverstein, R. L., and Febbraio, M. (2009) CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. Sci. Signal. 2, re3
10. Feng, J., Han, J., Pearce, S. F., Silverstein, R. L., Gotto, A. M., Jr., Hajjar, D. P., and Nicholson, A. C. (2000) Induction of CD36 expression by oxidized LDL and IL-4 by a common signaling pathway dependent on protein kinase C and PPAR-γ. J. Lipid Res. 41, 688–696
11. Berry, A., Balard, P., Coste, A., Olagnier, D., Lagane, C., Authier, H., Benoit-Vical, F., Lepert, J. C., Séguela, J. P., Magnaval, J. F., Chambon, P., Metzger, D., Desvergne, B., Wahli, W., Auwerx, J., and Pipp, B. (2007) IL-13 induces expression of CD36 in human monocytes through PPARγ activation. Eur. J. Immunol. 37, 1642–1652
12. Yakubenko, V. P., Bhattacharjee, A., Pluskota, E., and Cathcart, M. K. (2011) αMβ2 integrin activation prevents alternative activation of human and murine macrophages and impedes foam cell formation. Circ. Res. 108, 544–554
13. Patwarro, M. (1994) Adhesion molecules mediating recruitment of monocytes to injured tissue. Immunobiology 191, 474–477
14. Cabodi, S., Di Stefano, P., Leaf Mdel, P., Tinnirello, A., Bisaro, B., Morello, V., Damiano, L., Aramuu, S., Repetto, D., Tornillo, G., and Defilippi, P. (2010) Integrins and signal transduction. Adv. Exp. Med. Biol. 674, 43–54
15. Yamamoto, S. (1992) Mammalian lipoxygenases. Molecular structures and functions. Biochem. Biophys. Acta 1128, 117–131
16. Wittwer, J., and Hersberger, M. (2007) The two faces of the 15-lipoxygenase in atherosclerosis. Prostaglandins Leukot. Essent. Fatty Acids 77, 67–77
17. Serhan, C. N., Chiang, N., and Van Dyke, T. E. (2008) Resolving inflammation. Dual anti-inflammatory and pro-resolution lipid mediators. Nat. Rev. Immunol. 8, 349–361
18. Folcik, V., A., Nivar-Aristy, R. A., Krajewski, L. P., and Cathcart, M. K. (1995) Lipoxygenase contributes to the oxidation of lipids in human atherosclerotic plaques. J. Clin. Invest. 96, 504–510
19. Cathcart, M. K., and Folcik, V. A. (2000) Lipoxygenases and atherosclerosis. Protection versus pathogenesis. Free Radic. Biol. Med. 28, 1726–1734
20. Kühn, H., Belkner, J., Zais, S., Färklenkemper, T., and Wohlfel, S. (1994) Involvement of 15-lipoxygenase in early stages of atherosclerosis. J. Exp. Med. 179, 1903–1911
21. Huang, S. J., Guh, J. Y., Hung, W. C., Yang, M. L., Lai, Y. H., Chen, H. C., and Chuang, L. Y. (1999) Role of the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) cascade in advanced glycation end-product-induced cellular mitogenesis in NRK-49F cells. Biochem. J. 342, 231–238
22. Roy, B., and Cathcart, M. K. (1998) Induction of 15-lipoxygenase expression by IL-13 requires tyrosine phosphorylation of Jak2 and Tyk2 in human monocytes. J. Biol. Chem. 273, 32023–32029
23. Roy, B., Bhattacharjee, A., Xu, B., Ford, D., Maizel, A. L., and Cathcart, M. K. (2002) IL-13 signal transduction in human monocytes. Phosphorylation of receptor components, association with Jaks, and phosphorylation/activation of Stats J. Leukoc. Biol. 72, 580–589
24. Xu, B., Bhattacharjee, A., Roy, B., Xu, H. M., Anthony, D., Frank, D. A., Feldman, G. M., and Cathcart, M. K. (2003) Interleukin-13 induction of 15-lipoxygenase gene expression requires p38 mitogen-activated protein kinase-mediated serine 727 phosphorylation of Stat1 and Stat3. Mol. Cell. Biol. 23, 3918–3928
25. Conrad, D. J., Kuhn, H., Mulkins, M., Highland, E., and Sigal, E. (1992) Specific inflammatory cytokines regulate the expression of human monocyte 15-lipoxygenase. Proc. Natl. Acad. Sci. U.S.A. 89, 217–221
26. Nasser, G. M., Morrow, J. D., Roberts, L. J., 2nd, Lakis, F. G., and Badr, K. F. (1994) Induction of 15-lipoxygenase by interleukin-13 in human blood monocytes. J. Biol. Chem. 269, 27631–27634

JANUARY 25, 2013 • VOLUME 288 • NUMBER 4
Regulation of CD36 Expression by αMβ2 Integrin

27. Bhattacharjee, A., Xu, B., Frank, D. A., Feldman, G. M., and Cathcart, M. K. (2006) Monocyte 15-lipoxygenase expression is regulated by a novel cytosolic signaling complex with protein kinase Cδ and tyrosine-phosphorylated Stat3. J. Immunol. 177, 3771–3781

28. Bhattacharjee, A., Pal, S., Feldman, G. M., and Cathcart, M. K. (2011) Hck is a key regulator of gene expression in alternatively activated human monocytes. J. Biol. Chem. 286, 36709–36723

29. Wang, L. H., Yang, X. Y., Kirken, R. A., Resau, J. H., and Farrar, W. L. (2000) Targeted disruption of stat6 DNA binding activity by an oligonucleotide decoy blocks IL-4-driven TH2 cell response. Blood 95, 1249–1257

30. Huang, J. T., Welch, J. S., Ricote, M., Binder, C. J., Willson, T. M., Kelly, C., Witztum, J. L., Funk, C. D., Conrad, D., and Glass, C. K. (1999) Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase. Nature 400, 378–382

31. Podrez, E. A., Febrario, M., Sheibani, N., Schmitt, D., Silverstein, R. L., Hajjar, D. P., Cohen, P. A., Frazier, W. A., Hoff, H. F., and Hazen, S. L. (2000) Macrophage scavenger receptor CD36 is the major receptor for LDL modified by monocyte-generated reactive nitrogen species. J. Clin. Invest. 105, 1095–1108

32. Pluskota, E., Szpak, D., Ballantyne, C. M., Smith, J., Izem, L., Morton, R., Plow, E. F. (2010) CD11b Delays Development of Early Atherosclerotic Lesions in Female ApoE−/− Mice. ATVB poster, ePosters