Adipogenic differentiating agents regulate expression of fatty acid binding protein and CD36 in the J744 macrophage cell line

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Abstract **Adipocyte fatty acid binding protein (aP2) is a key mediator of intracellular transport and metabolism of fatty acids. Its expression during adipocyte differentiation is regulated through the actions of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα). Macrophages also express aP2, and the lack of macrophage aP2 significantly reduces atherosclerotic lesion size in hypercholesterolemic mice. We investigated the regulation of expression of macrophage aP2 and CD36, a fatty acid membrane binding protein and scavenger receptor, in response to the adipogenic agents isobutylmethylxanthine (IBMX), insulin, and dexamethasone, a combination of agents shown to induce fibroblast-to-adipocyte differentiation. Treatment of J774 macrophages with adipogenic agents significantly induced aP2 mRNA expression, while CD36 expression was inhibited. Dexamethasone was essential and sufficient to induce aP2 expression, and insulin had a synergistic effect. However, IBMX antagonized induced-aP2 expression. aP2 protein expression and [^{14}C]oleic acid uptake by macrophages were also increased by dexamethasone. Unlike what occurs in adipocytes, adipogenic agents had mixed effects on the expression of PPARγ and C/EBPα in macrophages. Our data demonstrate differences in the regulation of aP2 in adipocytes and macrophages and show that macrophage aP2 expression by adipogenic agents is independent of the PPARγ and/or C/EBPα signaling pathway.**—Sun, L., A. C. Nicholson, D. P. Hajjar, A. M. Gotto, Jr., and J. Han. **Adipogenic differentiating agents regulate expression of fatty acid binding protein and CD36 in the J744 macrophage cell line.** ***J. Lipid Res.*** **2003.** 44: **1877–1886.**

Supplementary key words adipogenic agents • adipocyte • CCAAT/enhancer

Fatty acid binding proteins (FABPs) are of a family of low-molecular-weight cytoplasmic proteins involved in intracellular transport and metabolism of fatty acids (1, 2). Two FABPs, adipocyte FABP [ALBP or adipocyte fatty acid binding protein (aP2)] and keratinocyte lipid binding protein, are expressed by adipocytes. aP2 is the predominant FABP, with a much greater expression level and higher affinity for most long-chain fatty acids (LCFAs) (3, 4). Fatty acids are taken up by adipocytes either passively by simple diffusion across the membrane (dependent on concentration and pH gradient or charge), or actively by transport proteins imbedded in the membrane. They are then sequestered by aP2 in the cytoplasm and shuttled back and forth from the adipocyte membrane to cellular organelles or to other proteins. aP2 also provides a shuttle system during the release and transport of fatty acids occurring in lipolysis/energy release and in lipogenesis/energy storage (1, 2).

aP2 is abundantly expressed in adipocytes and may represent as much as 1–3% of the intracellular soluble proteins. Its expression is highly regulated during adipocyte differentiation, and it is regarded as a marker of terminal cell differentiation (3–7). In vitro, adipocyte differentiation and aP2 expression are routinely induced by a combination of dexamethasone, insulin, and isobutylmethylxanthine (IBMX). In this process, dexamethasone is the primary adipogenic factor while insulin and IBMX play synergistic roles. In confluent preadipocytes, CCAAT/enhancer binding protein (C/EBP)β and C/EBPδ are induced primarily by dexamethasone and form a C/EBPβ/δ heterodimer, leading to peroxisome proliferator-activated receptor (PPAR)γ and C/EBPα activation. The activated PPARγ and C/EBPα cross-regulate each other to maintain their high expression. PPARγ and C/EBPα either alone or in cooperation induce the expression of adipocyte-related proteins and enzymes, such as adipsin, CD36, glycerol-3-phosphate dehydrogenase (GPDH), aP2, and insulin-dependent glucose transporter. All these products are involved in creating and maintaining the adipocyte phenotype. However, activation of PPARγ by its ligands

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Abbreviations: aP2, adipocyte fatty acid binding protein; C/EBPα, CCAAT/enhancer binding protein α; IBMX, isobutylmethylxanthine; OxLDL, oxidized LDL; PPARγ, peroxisome proliferator-activated receptor γ.

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alone also leads to adipocyte differentiation and aP2 expression (8–13).

Adipocytes and macrophages share a number of common features, including the ability to phagocytize and kill microorganisms and to secrete cytokines such as tumor necrosis factor α (TNFα) and interleukin-1 (14). Critical transcription factors in adipocytes involved in regulating expression of cytokines, inflammatory molecules, and fatty acid transporters are also expressed and have similar biologic roles in macrophages. For example, activation of PPARγ, a member of the nuclear-receptor superfamily of ligand-activated transcription factors, is associated with differentiation of both types of cells. In adipocytes, PPARγ regulates adipocyte development and glucose homeostasis. In macrophages, PPARγ regulates expression of inflammatory genes and is involved in the development of atherosclerotic lesions (15, 16).

CD36 is a common molecule expressed by both adipocytes and macrophages. It is a scavenger receptor that binds and internalizes oxidized LDL (OxLDL) in macrophages (17). CD36 also functions as an LCFA transporter to facilitate the uptake of LCFAs in adipocytes (18–20). CD36 expression is up-regulated by PPARγ during the differentiation of both types of cells (18, 21).

aP2 is also expressed by both adipocytes and macrophages. Although its action in macrophages needs to be fully defined, it is highly expressed in macrophages within atherosclerotic lesions of hypercholesterolemic mice. Lack of aP2 expression, particularly the lack of macrophage aP2 expression, significantly protects apolipoprotein E (apoE)-null mice from high-fat diet-induced atherosclerotic lesions, indicating the potential importance of macrophage aP2 in atherosclerotic lesion development (22–24). Oxidized LDL, PPARγ ligands, and PMA have been reported to increase monocyte/macrophage aP2 expression (25, 26). However, little else is known regarding the regulation and function of aP2 in macrophages. We have previously evaluated the regulation of macrophage aP2 in response to HDL and have shown that its expression is dependent on PPARγ and the extent of PPARγ phosphorylation (27). We and other researchers have also demonstrated that expression of CD36 in response to oxidized lipids, IL-4, and M-CSF is also mediated by PPARγ (28). In order to further determine mechanisms of aP2 and CD36 regulation in macrophages and to explore potential similarities and differences with the better defined regulatory pathways involved in the induction of aP2 expression in adipocytes, we evaluated the effects of adipogenic agents on the expression of aP2 and CD36 in macrophages. Our data demonstrate that while aP2 is induced in both cell types in response to adipogenic agents, expression of CD36 is inhibited.

EXPERIMENTAL PROCEDURES

Materials

All chemicals were purchased from Sigma-Aldrich, St. Louis, MO. Dexamethasone and IBMX were dissolved in ethanol and DMSO, respectively. Insulin was reconstituted according to the manufacturer's instruction. Anti-PPARγ and anti-C/EBPα antibodies were purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA. Rabbit anti-aP2 serum was generated by immunization with a murine aP2 peptide of DGKSTTIKRKRDGDKLV (29) by our lab.

Cell culture

All cell lines were purchased from American Type Culture Collection, Rockville, MD. J774 cells, a murine macrophage cell line, were cultured in RPMI 1640 medium containing 10% (v/v) fetal calf serum, 50 μg/ml of penicillin/streptomycin, and 2 mM glutamine. Cells were used to conduct experiments when they reached ~90% confluence. All treatments were performed in serum-free medium.

3T3-L1 cells, a murine fibroblast cell line, were cultured in 100 mm petri dishes with DMEM medium containing 10% fetal calf serum, 50 μg/ml of penicillin/streptomycin, and 2 mM glutamine. Cells were treated at the initial confluence within complete medium. All control cells received the corresponding solvent(s) treatments.

Extraction of total RNA, isolation of poly(A)$^+$ RNA, and Northern blotting

Cells were lysed in RNAzol™ B (Tel-Test, Inc., Friendswood, TX), chloroform extracted, and total cellular RNA was precipitated in isopropanol. After washing with 80% and 100% ethanol, the dried pellet of total RNA was dissolved in distilled water and quantified. The poly(A)$^+$ RNA was purified from ~100 μg total RNA by using PolyAT® tract mRNA Isolation System III (Promega, Madison, WI).

Poly(A)$^+$ RNA was loaded on 1% formaldehyde agarose gel. After electrophoresis, poly(A)$^+$ RNA was transferred to a Zeta-probe® Genomic Tested Blotting Membrane (Bio-Rad Laboratories, Richmond, CA) in 10× SSC by capillary force for overnight. The blot was ultraviolet crosslinked for 2 min, then prehybridized with Hybrisol™ I (Oncor, Inc., Gaithersburg, MD) for 30 min before the addition of ^{32}P randomly primed labeled probe for mouse aP2, CD36, or glyceraldehyde-3-phosphate dehydrogenase separately. After overnight hybridization, the membrane was washed for 2 × 20 min with 2× SSC and 0.2% SDS, and for 2 × 20 min with 0.2× SSC and 0.2% SDS at 55°C. The blot was autoradiographed by exposure to an X-ray film (Biomax™ MR, Kodak, Rochester, NY). The template DNA for aP2 was generated by RT-PCR based on the published sequences (29). The 5′- and 3′- sequences of oligonucleotides used were GATGCCTTTGTGGGAACC (307–325 on exon 1) and AACTCTTGTGGAAGTCACG (28–47 on exon 4), respectively. The size of PCR product was 377 bp. The probe for mouse CD36 is an *Nsi*I-*Bgl*II digest (base pairs 193–805).

Fatty acid binding assay

Macrophages were cultured in 12-well plates. After treatment, the cultured medium was aspirated and cells were washed once with incubation buffer [125 mM NaCl, 2.6 mM KCl, 5.7 mM Na_2HPO_4, 1.2 mM KH_2PO_4, 10 mM HEPES, 5.5 mM glucose (pH 8.5)]. [^{14}C]oleic acid working solution (300 nM, 1.76 μCi/ml [^{14}C]oleic acid and 300 nM BSA in incubation buffer) was added to cells and incubated for the times indicated followed by immediate removal of binding solution. Cells were washed twice with incubation buffer, then lyzed by addition of 1 ml 0.2 N NaOH and incubation for 30 min at 37°C. The protein content and radioactivity in lysate were determined.

Western analysis of aP2 protein expression

After treatment, macrophages were washed twice with cold PBS, then scraped and lysed in ice-cold lysis buffer [50 mM Tris

(pH 7.5), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxychlorate, 1 mM phenylmethylsulfonyl fluoride (PMSF), 50 mM sodium fluoride, 1 mM sodium orthvanadate, 50 μg/ml aprotinin, and 50 μg/ml leupeptin]. Lysate was sonicated for 20 cycles, and then centrifuged for 15 min at 4°C. The supernatant was transferred to a new test tube. Proteins were separated on a 12% SDS-PAGE and then transferred onto nylon-enhanced nitrocellulose membrane. The membrane was blocked with a solution of TBS (20 mM Tris, 139 mM NaCl) containing 5% fat-free milk for 1 h, then incubated with rabbit anti-aP2 serum for 2 h at room temperature followed by washing for 3 × 10 min with TBS-T (0.5% Tween 20) buffer. The blot was then reincubated with horseradish peroxidase-conjugated goat anti-rabbit IgG in TBS for another hour at room temperature. After washing 3 × 10 min with TBS-T, the membrane was incubated for 1 min in a mixture of equal volumes of Western blot chemiluminescence reagents 1 and 2. The membrane was then exposed to film before development.

Extraction of nuclear protein and Western analysis of PPARγ and C/EBPα

After treatment, cells were washed twice with cold PBS and then resuspended in 400 μl of cold buffer A [10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF]. After 15 min incubation on ice, the cell suspension was added to 25 μl of 10% NP-40 and vortexed vigorously for 10 s. The supernatant was removed afterwards and spun for 30 s at 13,000 rpm. The pellet was resuspended in 100 μl cold buffer C [20 mM HEPES (pH 7.91), 400 mM KCl, 1 mM EDTA, 1 mM EGTA, freshly added 1 mM DTT, 1 mM PMSF, 1 μg/ml leupeptin, and 10 μg/ml aprotinin] and kept for 15 min on ice. The mixture was spun for 15 min at 13,000 rpm with a microfuge and the supernatant was collected as the nuclear proteins.

Nuclear proteins (40 μg) from each sample were loaded on SDS-PAGE gels and separated by electrophoresis, then transferred onto nylon-enhanced nitrocellulose membranes. PPARγ and C/EBPα were determined by Western blot with anti-PPARγ and -C/EBPα antibodies, respectively.

Data analysis

All the data were repeated at least three times, and representative results were presented. In the fatty acid binding assay, the data were analyzed by paired Student's *t*-test.

RESULTS

Adipogenic agents induce macrophage expression of aP2 but inhibit CD36

To investigate the effect of adipogenic agents on macrophage aP2 expression, we cultured J774 cells in serum-free medium containing IBMX (0.5 mM), insulin (0.8 μM), and dexamethasone (2.5 μM) for up to 4 days. These concentrations were chosen based on the demonstrated effect of this mixture on adipocyte differentiation (10). As in adipocytes, aP2 mRNA was markedly induced in macrophages. Induction was time dependent with the appearance of aP2 by day 1 and peak expression at day 4 (**Fig. 1**). aP2 mRNA was undetectable in untreated cells. In adipocytes, adipogenic agents induced both aP2 and CD36 gene expression (30). We next evaluated the effect of adipogenic agents on CD36 expression in macrophages using the same treatment protocol. In contrast to the increase observed in aP2 expression, CD36 was down-regulated in a time-dependent manner by treatment with IBMX, insulin, and dexamethasone (Fig. 1). Similar effects of adipogenic agents on aP2 and CD36 expression were observed with PMA-differentiated THP-1 cells, a human monocytic cell line (data not shown).

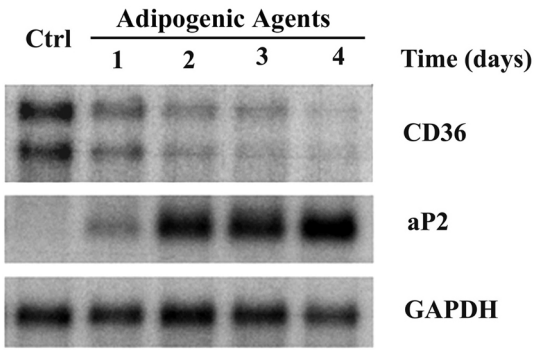

Fig. 1. Adipogenic agents [dexamethasone, insulin, and isobutylmethylxanthine (IBMX)] induce adipocyte fatty acid binding protein (aP2) and inhibit CD36 expression in macrophages. J774 macrophages were cultured in complete RPMI medium until ~90% confluence. Cells then were switched to serum-free medium and treated with insulin (0.8 μM), dexamethasone (2.5 μM), and IBMX (0.5 mM) on days 1 and 2. On days 3 and 4, the cells were treated with insulin (0.8 μM) alone. Total cellular RNA was extracted at the indicated times and used to isolate poly(A)$^+$ RNA. Macrophage aP2 and CD36 mRNA were analyzed by Northern blot as described in Experimental Procedures. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was detected for RNA loading and integrity.

We next determined the effect of each component of the adipogenic-differentiating combination on both macrophage and differentiating preadipocyte aP2 expression. We treated confluent J774 macrophages or confluent 3T3 fibroblasts with IBMX, insulin, and dexamethasone, as well as various combinations for 4 days to determine the contribution of each agent to CD36 and aP2 expression in these two cell types. In both macrophages and differentiating preadipocytes, dexamethasone alone was able to induce aP2 expression (**Fig. 2**). Both insulin and IBMX further enhanced aP2 expression in differentiating preadipocytes, suggesting that they played a synergistic role with the greater effect by IBMX. The combination of all three agents had the greatest effect on inducing aP2 expression in fibroblasts (Fig. 2B). Although insulin was still able to enhance aP2 expression in macrophages, IBMX strongly inhibited the induction of aP2 expression by dexamethasone or dexamethasone plus insulin (Fig. 2A). In differentiating preadipocytes, IBMX, insulin, dexamethasone, and their combinations regulated CD36 expression similarly to aP2 (Fig. 2B). However, in macrophages, changes of CD36 expression were opposite those of aP2 expression caused by adipogenic agents (Fig. 2A). For example, IBMX inhibited aP2 expression, but induced CD36 expression significantly. In addition, dexamethasone or dexamethasone plus insulin induced aP2 expression while suppressing CD36 expression in macrophages (Fig. 2A).

To determine if induction of macrophage aP2 mRNA expression correlated with increased aP2 protein expres-

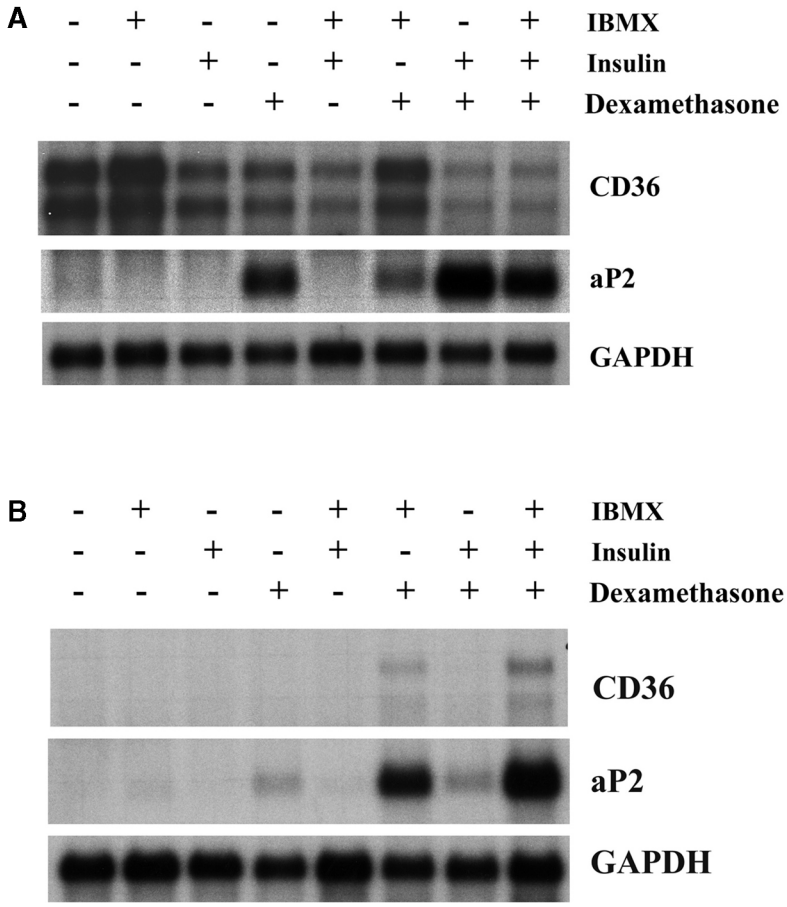

Fig. 2. Different effects of dexamethasone, insulin, and IBMX on aP2 and CD36 expression in macrophages and preadipocytes. A: Confluent J774 macrophages were treated with dexamethasone (2.5 μM), insulin (0.8 μM), and IBMX (0.5 mM), or various combinations, as indicated, in serum-free medium for 4 days. Total RNA was extracted and used to isolate poly(A)$^+$ RNA. aP2 and CD36 mRNA levels were determined by Northern blot as described in Experimental Procedures. B: Mouse 3T3 preadipocytes were cultured in complete DMEM medium containing 10% fetal calf serum, 50 μg/ml of penicillin-streptomycin, and 2 mM glutamine. At the first day of confluence, the cells were treated as in A and harvested after 4 days. CD36 and aP2 mRNA levels were detected by Northern blot.

sion, we evaluated aP2 protein expression by Western blot analysis. Consistent with the changes observed in aP2 mRNA, aP2 protein was induced by dexamethasone alone and further by cotreatment with insulin (**Fig. 3A**). However, aP2 protein levels in macrophages were much lower than those observed in adipocytes.

Because aP2 functions as fatty acid binding protein in cytoplasm and dexamethasone was the primary inducer for macrophage aP2 expression, we determined the effect of dexamethasone on fatty acid uptake by macrophages. After 4 days of treatment with dexamethasone, macrophages were used to conduct the fatty acid binding assay using ^{14}C-labeled oleic acid. Dexamethasone significantly increased the uptake of [^{14}C]oleic acid (~2-fold) by macrophages (Fig. 3B).

Dexamethasone is necessary and sufficient to induce macrophage aP2 expression

The role of dexamethasone in inducing macrophage aP2 expression was further characterized. Macrophages were treated with different concentrations of dexamethasone for 4 days. Dexamethasone up-regulated macrophage aP2 expression in a dose-dependent manner (**Fig. 4A**). A time-course study revealed that induction of macrophage aP2 expression by dexamethasone (2.5 μM) was time dependent. The earliest expression of aP2 mRNA was observed after 1 day of exposure to dexamethasone and continued for 6 days (Fig. 4B).

To test the essential role of dexamethasone in maintaining expression of macrophage aP2, macrophages were pretreated with dexamethasone (2.5 μM) for 3 days. aP2 mRNA was evaluated in cells cultured with or without dexamethasone for an additional 3 days. aP2 expression was observed only in cells maintained in dexamethasone (**Fig. 5**). When dexamethasone was withdrawn, the aP2 expression was maintained for only 1 day and then expression dropped so dramatically that it was barely detectable at the third day following removal of dexamethasone (Fig. 5).

Insulin is synergistic with, while IBMX inhibits, macrophage aP2 expression induced by dexamethasone

To evaluate the effects of insulin on aP2 expression, macrophages were treated for 4 days with either dexamethasone alone, insulin alone, or dexamethasone plus insulin. Insulin alone was not able to induce macrophage aP2 expression but could enhance aP2 expression when used in combination with dexamethasone (**Fig. 6A**). However, macrophage aP2 expression was enhanced by cotreatment with insulin and dexamethasone (Fig. 6A). This synergistic effect was independent of time of treatment and persisted from day 1 until the last day of treatment (day 4). The concentration of insulin affected aP2 expression when combined with a fixed concentration of dexamethasone. In the presence of dexamethasone (2.5 μM), a low concentration of insulin (≤50 nM) had a moderately additive effect on aP2 induction by dexamethasone. A high

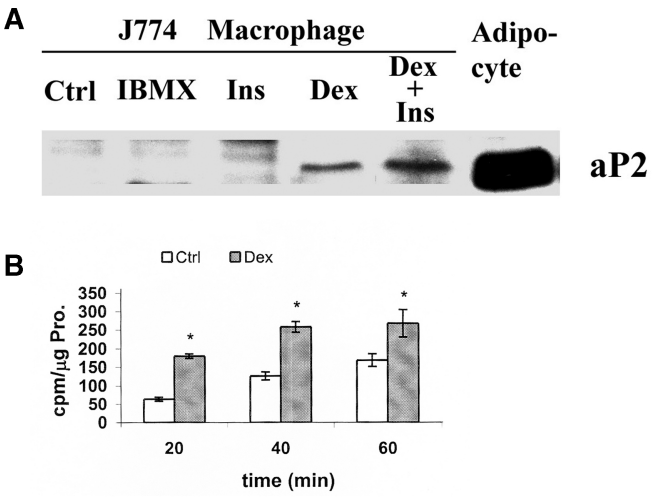

Fig. 3. Dexamethasone increases macrophage aP2 protein expression and fatty acid binding capacity. A: Confluent J774 cells were treated with IBMX (0.5 mM), insulin (0.8 μM), dexamethasone (2.5 μM), or dexamethasone plus insulin in serum-free medium for 4 days. Whole-cell lysate protein (40 μg) from each sample was loaded on 12% SDS-PAGE and separated by electrophoresis. aP2 protein expression was analyzed by Western blot as described in Experimental Procedures. Adipocyte differentiation was completed by incubation of confluent fibroblasts for 2 days in complete DMEM medium containing dexamethasone (2.5 μM), insulin (0.8 μM), and IBMX (0.5 mM), then 2 days with insulin (0.8 μM), and an additional 2 days in complete medium. Adipocyte lysate (40 μg) was used as positive control. B: J774 cells were cultured in 12-well plates until confluence then treated with dexamethasone (2.5 μM) for 4 days. After washing once with buffer, cells were cultured with [^{14}C]oleic acid (300 nM) as described in Experimental Procedures. The assay was completed in quadruplicate and data were analyzed by Student's *t*-test. Error bars indicate SD. * Significantly different from control at $P < 0.05$.

concentration of insulin (≥200 nM) had a more marked effect on aP2 expression (Fig. 6B).

IBMX is routinely used with dexamethasone and insulin to differentiate adipocytes in culture. IBMX enhances expression of aP2 in differentiating preadipocytes treated with dexamethasone or dexamethasone plus insulin (Fig. 2B). However, in macrophages, IBMX suppressed aP2 expression induced by dexamethasone or dexamethasone plus insulin (Fig. 2A). To gain more insight into the effects of IBMX on macrophage aP2 expression, we studied the kinetics of inhibition by IBMX. IBMX was added to cells simultaneously with dexamethasone. At each time point, the effect of dexamethasone on macrophage aP2 expression was abolished by IBMX (**Fig. 7A**). IBMX inhibited macrophage aP2 expression induced by dexamethasone or dexamethasone plus insulin in a dose-dependent manner (Fig. 7B).

In addition to blocking the initiation of macrophage aP2 expression, IBMX was also able to significantly decrease the high level of dexamethasone-induced aP2 gene expression. Macrophages were pretreated with dexamethasone (2.5 μM) for 4 days and then treated with IBMX (0.5 mM) for an additional 4 days in the continuous presence of dexamethasone. Compared with dexamethasone alone, added IBMX significantly decreased the induced aP2 levels (**Fig. 8**).

Regulation of macrophage aP2 expression by adipogenic agents is independent of PPARγ and C/EBPα

To determine potential mechanisms by which adipogenic agents modulate expression of macrophage aP2 and CD36, we evaluated expression of PPARγ and C/EBPα, nuclear transcription factors that regulate expression of aP2 and CD36 in adipocytes. We evaluated expression of PPARγ and C/EBPα following incubation with the combination of dexamethasone, insulin, and IBMX, or with individual components of this mixture. Western blot analysis demonstrated that macrophage PPARγ expression was induced by all treatments at day 1. In addition, dexamethasone and insulin also increased expression of phosphorylated PPARγ, the inactive form of PPARγ (**Fig. 9A**). Expression of C/EBPα was unaltered by treatments at day 1 (Fig. 9A). After 5 days, all treatments but insulin alone increased C/EBPα expression (Fig. 9B), and all but dexamethasone increased PPARγ expression. Therefore, changes of PPARγ and/or C/EBPα expression could not correlate with that of aP2 expression induced by adipogenic agents in macrophages.

DISCUSSION

Our data demonstrate that a combination of dexamethasone, insulin, and IBMX can induce expression of macrophage aP2 while inhibiting CD36 expression. The mechanisms by which adipogenic agents regulate macrophage aP2 and CD36 induction are unclear, but expression of PPARγ/C/EBPα protein does not correlate in a temporal manner with expression of aP2 and CD36.

Although aP2 is thought to play an important role in solubilization and intracellular trafficking of fatty acids, aP2-deficient mice are developmentally and metabolically normal. Deficiency of aP2 results in the prevention of dietary, obesity-induced insulin resistance or diabetes, the reduction of TNFα in adipose tissue, and lipolysis and the elevation of cellular fatty acid levels (31, 32). Deficiency of macrophage aP2 significantly protects apoE-null mice from high-fat diet-induced atherosclerotic lesions (23, 24).

aP2 expression is significantly induced in both differentiating preadipocytes and macrophages by dexamethasone, insulin, and IBMX; however, our data illustrate significant differences in these two cell types. Induction of aP2 in preadipocytes is associated with cell differentiation, and aP2 is a marker of differentiated adipocytes. In J774 macrophages, induction of aP2 expression is reversible. Withdrawal of dexamethasone, the primary activator, can cause a rapid decline in aP2 expression. The effects of adipogenic agents on macrophages were not related to their effects on macrophage differentiation. We did not observe significant changes in CD14 or CD68 (two proteins associated with macrophage differentiation) expression in J774 macrophages treated with individual or combinations of adipogenic agents (data not shown). In addition, we observed no morphological alterations in macro-

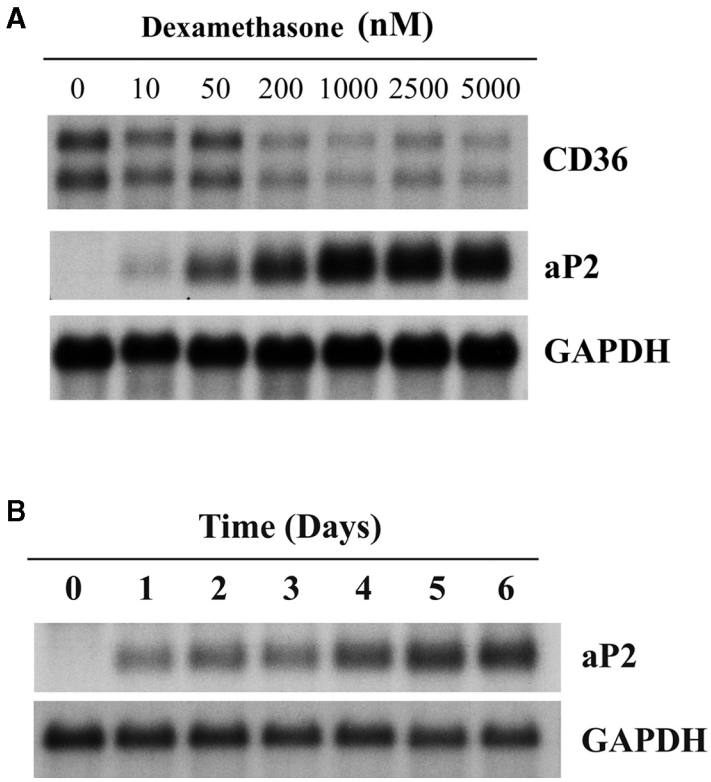

Fig. 4. Dexamethasone induces macrophage aP2 expression in a concentration- and time-dependent manner. A: Confluent macrophages were treated with dexamethasone at the indicated concentrations for 4 days in serum-free medium. Expression of aP2 and CD36 were analyzed by Northern blot as described in Experimental Procedures. B: Confluent macrophages were switched to serum-free medium and treated with dexamethasone (2.5 μM) for the indicated times. aP2 mRNA expression was evaluated by Northern blot.

phages treated with dexamethasone for 4 days or longer (up to 20 days), and dexamethasone did not induce expression of two other genes linked to adipogenesis, adipsin and GPDH, in macrophages (data not shown).

CD36 is highly expressed by both macrophages and adipocytes. Expression of CD36, like aP2, is mediated through activation of PPARγ and C/EBPα in adipocytes. In monocyte/macrophages, ligand activation of PPARγ significantly up-regulates CD36 expression (21). Although aP2 and CD36 are coordinately regulated in adipocytes, aP2 and CD36 expression changed inversely in macrophages in response to adipogenic agents (Figs. 1, 2A, 4A, 6B). Expression of C/EBPα in macrophages did not correlate with expression of either CD36 or aP2 in macrophages in response to adipogenic agents (Fig. 9). Although activation of PPARγ in monocytes by both natural (15d-PGJ_2) and synthetic agonists (troglitazone and BRL-49653) modestly increased aP2 expression (25), our Western data demonstrate that changes of PPARγ/C/EBPα expression could not correlate with that of aP2 expression induced by adipogenic agents in macrophages. In addition, we cotreated J774 macrophages with dexamethasone and PPARγ agonists (troglitazone and PGJ2) and did not observe either a synergistic or an inhibitory effect of PPARγ agonists on induction of aP2 expression by dexamethasone (data not shown). Thus, it is unlikely that adipogenic agents regulate macrophage aP2 expression through PPARγ and/or C/EBPα.

Induction of aP2 expression by OxLDL in human THP-1 PMA-induced macrophages has been reported to involve activation of the nuclear factor (NF)-κB pathway (26). Dexamethasone is a lipophilic steroid hormone that enters cells by free diffusion across the lipid bilayer and functions through binding to an intracellular glucocorticoid receptor (33). We observed that it activated macrophage aP2 gene expression in both a time- and concentration-dependent manner. However, it is unlikely that dexamethasone modulates macrophage aP2 expression through NF-κB, because the glucocorticoid receptor has been shown to physically interact with NF-κB in vitro and

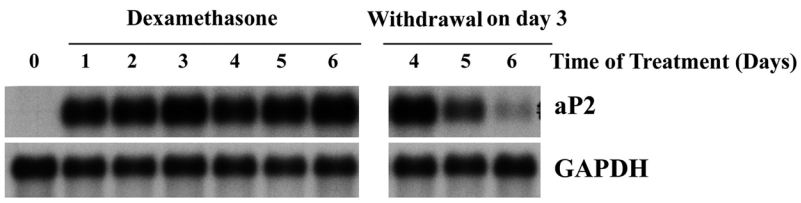

Fig. 5. Dexamethasone is essential for maintaining macrophage aP2 expression. Confluent macrophages were pretreated with dexamethasone (2.5 μM) for 3 days in serum-free medium. The cells were then cultured for an additional 3 days with or without dexamethasone. Total RNA was extracted at the indicated times, and aP2 mRNA was analyzed by Northern blot.

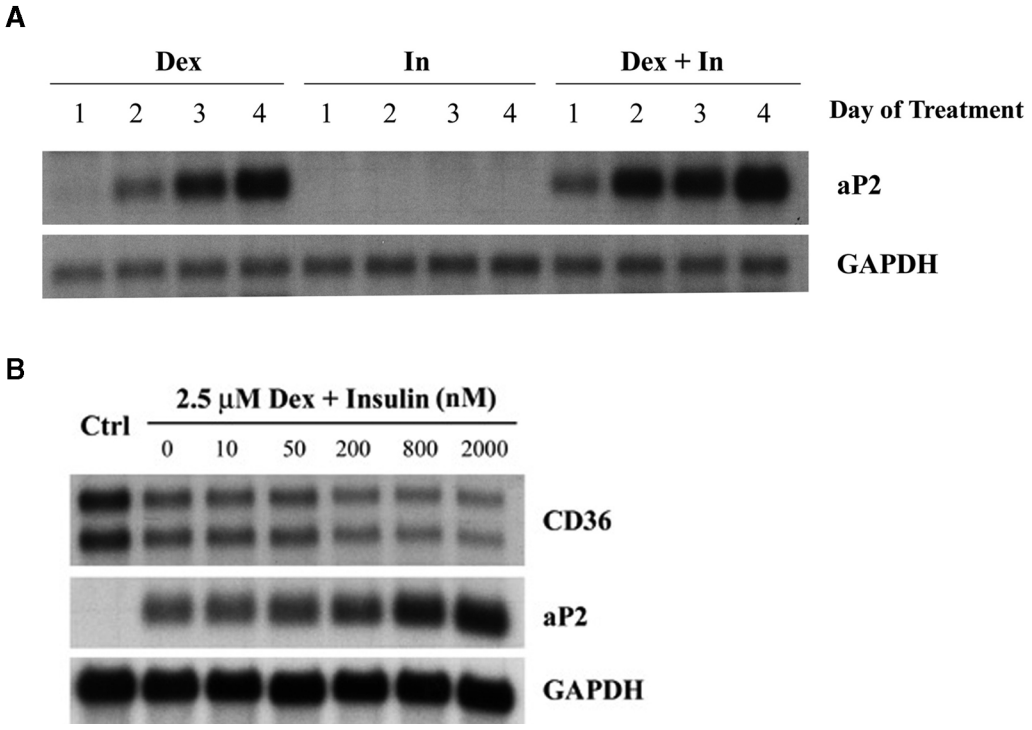

Fig. 6. Insulin acts synergistically with dexamethasone to induce macrophage aP2 expression. A: Confluent macrophages were treated with dexamethasone (2.5 μM), insulin (0.8 μM), or dexamethasone plus insulin for the times indicated. Cells were harvested to extract total RNA. aP2 expression was analyzed by Northern blot. B: Confluent macrophages were treated with insulin at the indicated concentrations in the presence of dexamethasone (2.5 μM) for 4 days. Expression of aP2 and CD36 was evaluated by Northern blot as above.

to inhibit NF-κB activity (33). The aP2 gene contains a glucocorticoid response element within the upstream regulatory region (34). Thus, glucocorticoids may directly regulate aP2 expression. Dexamethasone induces aP2 gene expression in both adipocytes and nonadipocytes (the 3T3-C2 nonpreadipocyte cell line and the differentiated myogenic C_2C_{12} cell line) (35). While induction of aP2 expression by fatty acids requires ongoing protein syn-

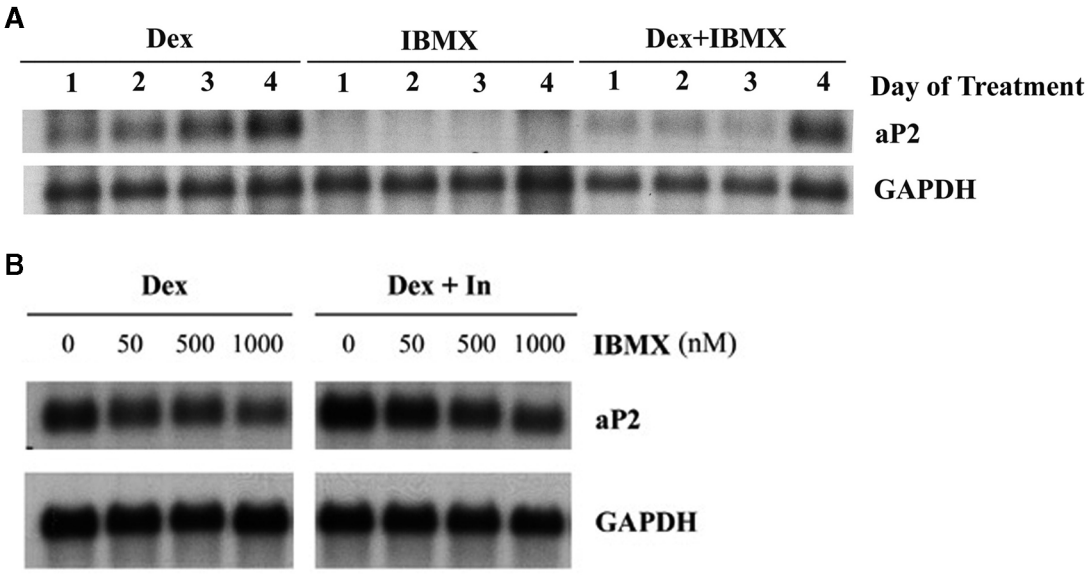

Fig. 7. IBMX inhibits macrophage aP2 expression induced by dexamethasone or dexamethasone and insulin. A: Confluent macrophages were treated with dexamethasone (2.5 μM), IBMX (0.5 mM), or dexamethasone plus IBMX. Cells were harvested at the indicated times. aP2 mRNA expression was evaluated by Northern blot. B: Confluent macrophages were treated with IBMX at the indicated concentrations in the presence of dexamethasone (2.5 μM) or dexamethasone (2.5 μM) plus insulin (0.8 μM) for 4 days. Expression of aP2 was determined by Northern blot as described in Experimental Procedures.

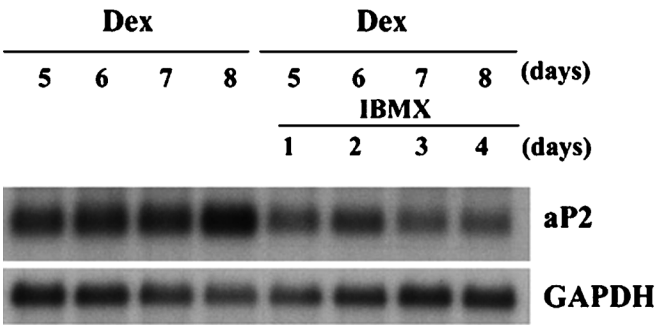

Fig. 8. IBMX decreases induced macrophage aP2 levels. After 4 days of treatment with dexamethasone (2.5 μM), macrophages maintained in culture with dexamethasone or dexamethasone plus IBMX (0.5 mM) for the times indicated. Total RNA was isolated and aP2 expression was analyzed by Northern blot.

thesis, dexamethasone induces aP2 gene expression in nonadipocyte cell lines in the presence of cycloheximide, suggesting that dexamethasone does not require the synthesis of any intermediate proteins (35).

IBMX increases cellular cAMP level by inhibiting phosphodiesterases. During adipogenesis, increased cAMP is able to stimulate C/EBPα-PPARγ and therefore increases aP2 expression (36–39). In macrophages, PPARγ-C/EBPα expression did not correlate with aP2 expression. However, this does not rule out a role for these transcription factors in the regulation of aP2 in macrophages, as we have only evaluated the coordinate expression of these transcription factors and not their activity. The mechanism by which insulin modulates macrophage aP2 expression is also unclear. To satisfy the physiological function of insulin to trigger lipogenesis/energy storage, more aP2 protein is needed to transport fatty acids for internalization and esterification. Insulin has been reported to phosphorylate MAP kinase, and MAP kinase in turn can phosphorylate a large number of target substrates (40, 41). MAP kinase-phosphorylated PPARγ inhibits adipocyte differentiation, aP2, and macrophage CD36 expression (42–44, 27, 45). Consistent with these findings, we observed increased phosphorylation of PPARγ and subsequent down-regulation of CD36 expression (Figs. 6B, 9). Regulation of macrophage aP2 expression by adipogenic agents did not correlate with PPARγ expression. Therefore, the increased phosphorylation of PPARγ by insulin may not contribute to the down-regulation of aP2 expression.

Taken together, our data demonstrate that adipogenic agents can regulate macrophage aP2 and CD36 expression. While the effect of adipogenic agents on aP2 expression is similar in adipocytes and macrophages, the effect on CD36 expression is not, with induction of expression in adipocytes and suppression of expression in macrophages. Although the role of aP2 in macrophages needs to be further defined, several lines of evidence have demonstrated the potential role of aP2 in atherosclerosis. It is highly expressed in macrophages within atherosclerotic lesions. Lack of aP2 expression significantly protects hypercholesterolemic mice from development of atherosclerotic lesions (22–24). Therefore, study of macrophage aP2 expression and regulation is of great importance. The role of CD36 in atherosclerosis has been clearly demonstrated. Atherosclerosis-prone mice (apoE$^{-/-}$) that lack CD36 expression (CD36$^{-/-}$) have markedly reduced atherosclerotic lesion size (17). Dexamethasone has been shown to decrease atherosclerotic lesion size in several animal models (46–49). However, the role of aP2 and CD36 in the response of atherosclerotic lesion inhibition by dexamethasone remains to be tested.

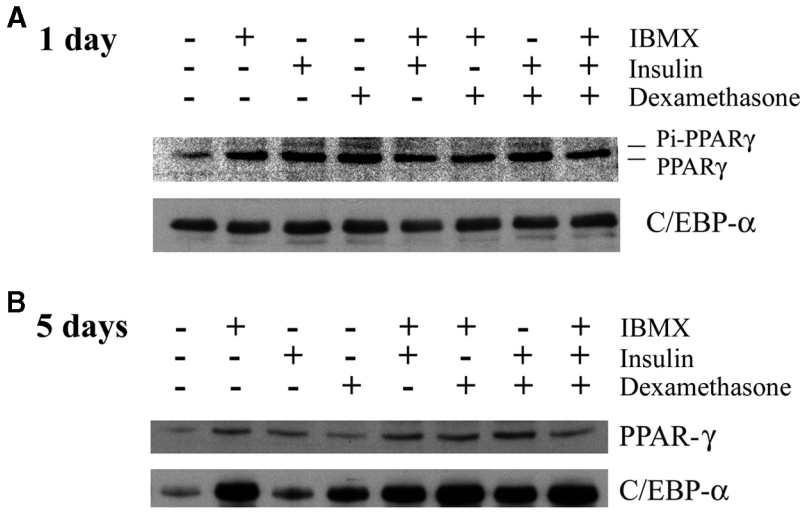

Fig. 9. Adipogenic agents regulate macrophage peroxisome proliferator-activated receptor (PPAR)γ/phospho-PPARγ and CCAAT/enhancer binding protein α (C/EBPα) protein expression. Confluent macrophages were treated in serum-free medium with IBMX (0.5 mM), insulin (0.8 μM), or dexamethasone (2.5 μM) alone or in combination as indicated. Following treatment (A: 1 day of treatment; B: 5 days of treatment), nuclear protein was extracted from cells as described in Experimental Procedures. Protein (40 μg/sample) was separated by electrophoresis, transferred to nitrocellulose membranes, and subjected to Western blot to determine expression of PPARγ/phospho-PPARγ and C/EBPα protein.

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