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Interleukin-6 and interleukin-15 are selectively regulated by lipopolysaccharide and interferon-γ in primary pig adipocytes

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Ajuwon, Kolapo M., Sheila K. Jacobi, Joanne L. Kuske, and Michael E. Spurlock. Interleukin-6 and interleukin-15 are selectively regulated by lipopolysaccharide and interferon-γ in primary pig adipocytes. *Am J Physiol Regul Integr Comp Physiol* 286: R547–R553, 2004. First published December 4, 2003; 10.1152/ajpregu.00585.2003.—3T3-L1 adipocytes express the lipopolysaccharide (LPS) receptor and respond to direct stimulation with the antigen by increasing the expression of inflammatory mediators. Activation of this receptor by its ligand in the macrophage causes the activation and translocation of nuclear factor-κB (NF-κB) to the nucleus where it regulates the expression of proinflammatory cytokines and other target genes. We investigated whether LPS could stimulate NF-κB translocation in primary pig adipocytes and regulate the expression and secretion of TNF-α and IL-6. LPS clearly induced the nuclear translocation of NF-κB and also upregulated ($P < 0.05$) the mRNA expression and secretion of IL-6 into the culture medium. An induction of TNF-α expression by LPS was not detected, but with extended incubation (8 h), there was a modest increase ($P < 0.09$) in the media concentration of this cytokine. Inhibition of either ERK1/2, PKC, or the inhibitory G protein (G_i) with U-0126, bisindolylmaleimide HCl, and pertussis toxin, respectively, blocked ($P < 0.05$) the increase in IL-6 expression caused by LPS. Because LPS administration in vivo increases circulating concentrations of IFN-γ, and because this cytokine also regulates multiple immune modulators in the adipocyte, we also determined whether IFN-γ regulates cytokine expression in primary adipocytes. Although the expression of IL-6 and TNF-α was unresponsive to IFN-γ, the expression of IL-15 was markedly upregulated ($P < 0.01$). Furthermore, the induction of IL-15 expression by IFN-γ was blocked by inhibition of PKC. These data indicate that NF-κB is responsive to LPS in the adipocyte and also identify key mediators of LPS-induced IL-6 expression. In addition, we provide novel evidence that IFN-γ targets the adipocyte to induce IL-15 expression, thus indicating a possible role for the adipocyte in the regulation of T-cell function and muscle metabolism during the innate immune response.

adipocyte; interleukin; interferon

THE MARKED SIMILARITY of cardiovascular disease in the pig with that in humans (11), coupled with similar linkages among hyperglycemia, dyslipidemia, and insulin resistance (22), indicates that the pig can serve as an important model for some diseases, including diabetes and the metabolic syndrome. The pig also provides a model system in which an abundance of primary adipocytes and myogenic cells can be recovered with ease for experimental needs. Accordingly, there is considerable need to delineate in porcine models specific pathways that impact energy homeostasis and insulin sensitivity.

The adipocyte produces a number of cytokines that exert metabolic and immunological activities locally and on cells in other tissues (reviewed by Ref. 6). Particularly intriguing are the recent major findings which show that the adipocyte is equipped at the molecular level to function in the innate immune response. First, Lin et al. (17) showed clearly that the 3T3-L1 adipocytes express the Toll-like receptors 2 and 4 (*Tlr-2* and *Tlr-4*), the latter one being the signaling receptor for bacterial LPS. Furthermore, this group showed that adipocytes respond to direct stimulation with LPS or TNF-α by upregulating IL-6 expression and *Tlr-2* synthesis, and TNF-α and IL-6 expression was upregulated by culturing with either of these cytokines alone. These findings are of considerable interest in light of the new paradigm in which adipocytes are viewed as immune cells and because of the metabolic actions of these cytokines, especially in relationship to insulin resistance (13, 14). Second, adipocytes produce several acute phase proteins (ACP, 25). Whereas the biological roles of these adipocyte-derived ACP are not yet clearly defined, it is most intriguing that some of them have recently been associated with the hyperglycemia of type II diabetes (18).

Although the adipocyte has now been linked directly to the innate immune response, the signaling pathway has not been studied in this cell, nor has it been determined whether the adipocyte expresses cytokines other than TNF-α and IL-6 in response to LPS. Accordingly, the objective of the study reported herein was threefold. First of all, we sought to confirm in part the findings of Lin et al. (17) in a primary adipocyte model and to determine whether LPS signaling in the adipocyte results in activation of NF-κB and enhanced expression of proinflammatory cytokines. Second, we wanted to identify critical steps in the LPS signaling pathway in the adipocyte through the use of selective inhibitors shown previously to regulate *Tlr-4* signaling in other cell types. Finally, we sought to test the hypothesis that IFN-γ acts directly on the adipocyte to regulate the expression of immune modulators independent of the effect of LPS on the adipocyte.

MATERIALS AND METHODS

Adipocyte isolation. Adipocytes were isolated from the subcutaneous fat depot of male castrate pigs weighing ~100 kg by collagenase digestion as described previously (19, 27, 28). Briefly, the isolated cells were diluted to approximate 20% cell suspensions with DMEM (Sigma, St. Louis, MO) containing 3% fatty acid-free serum albumin. The cell suspensions were gassed initially (and at 2-h intervals) with a mixture of air and CO_2 and were incubated in a gyratory floor incubator at 37°C for the selected duration. To terminate the reaction, the adipocytes were allowed to float on the surface, and the medium was carefully aspirated from the bottom of the vial for assays. The cells were used for RNA isolation or for preparation of nuclear

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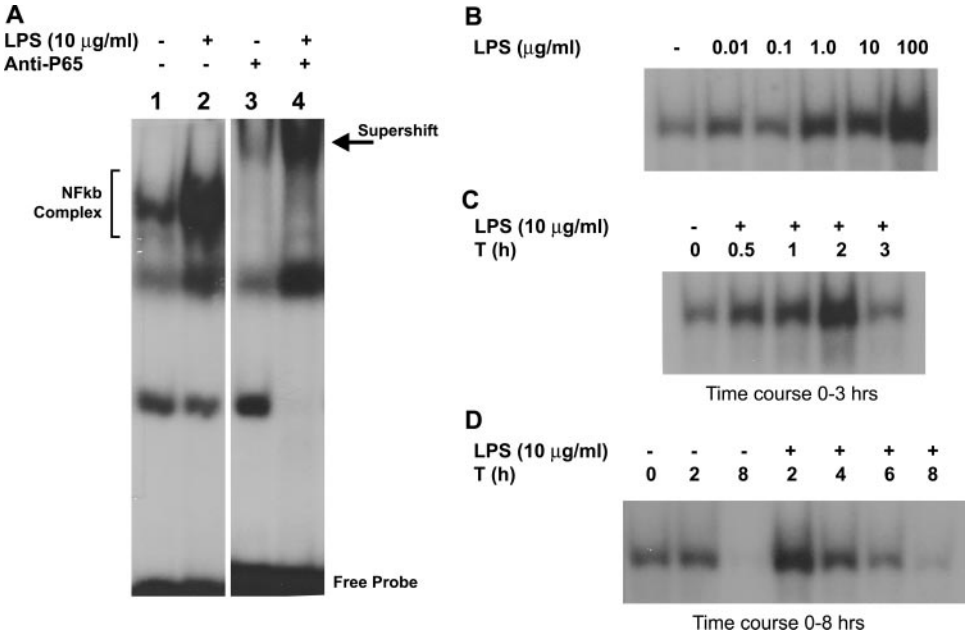

Fig. 1. Evidence of direct response of primary porcine adipocytes to LPS. *A*: adipocytes were isolated and cultured for 2 h as described in MATERIALS AND METHODS in the presence or absence of LPS (10 μg/ml). Experiments were performed with samples from at least 3 different pigs with similar results. P-65, anti P-65 NF-κB antibody. *B*: LPS activates NF-κB in a dose-dependent manner. Adipocytes were treated with LPS at the concentrations indicated (0.01–100 μg/ml) for 2 h before the gel shift procedure was performed on the nuclear extracts. Adipocytes were incubated with 10 μg/ml LPS for up to 3 h (*C*) or up to 8 h (*D*).

extracts for electrophoretic mobility shift assays. The experiments were repeated at least three times with cells obtained from different pigs. Within each experiment (pig), each treatment was replicated at least three times. Adipocytes were pretreated for 1–2 h with enzyme inhibitors before the addition of LPS to the medium. Inhibitors of MEK (U-0126 and PD-98059), p38 (SB-203580), JNK (SP-600125), PKC (bisindolylmaleimide HCl), and G_i (pertussis toxin) were obtained from Calbiochem (La Jolla, CA). The p44/42 MAP kinase inhibitors U-0126 and PD-98059 were used at concentrations 15 and 20 μM, respectively. SB-303580 and SP-600125 were used at a final concentration of 10 μM. Bisindolylmaleimide HCl and pertussis toxin were used at concentrations 20 μM and 1 μg/ml, respectively.

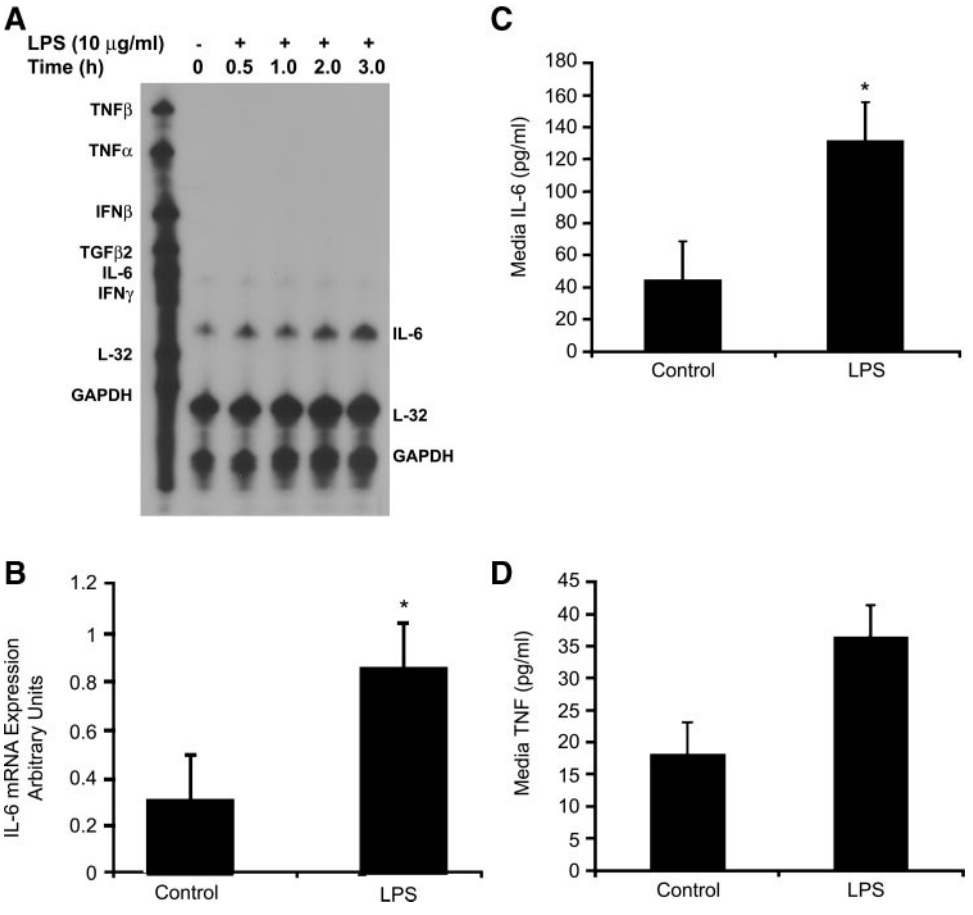

Fig. 2. LPS induces TNF-α secretion and IL-6 secretion and expression. *A*: adipocytes were treated with 10 μg/ml LPS, and the expression of IL-6 mRNA was determined at 0.5, 1, 2 and 3 h by RNase protection assay. The image is representative of 3 replicate experiments. TGFβ2, transforming growth factor-β2. L-32, ribosomal L32 protein. *B*: adipocytes were treated for 3 h with LPS (10 μg/ml), and the induction of IL-6 mRNA was determined by RNase protection assay. Data are means ± SE from 10 different pigs. *Significance at $P < 0.05$. Adipocytes were cultured with LPS (10 μg/ml) for 3 h (*C*) or 8 h (*D*), after which media were assayed by ELISA for IL-6 and TNF-α, respectively. *Significance at $P < 0.05$. Data presented represent means ± SE of results from 5 different pigs.

Electrophoretic mobility shift and supershift assays. Nuclear proteins were extracted from primary porcine adipocytes (minimum of 5×10^5 cells per treatment) according to the method of Natarajan et al. (20) with slight modifications. At the conclusion of the incubation, the cells were rinsed three times with prewarmed PBS before being suspended in 400 μl of ice-cold lysis buffer [10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA,0.1 mM EGTA, 0.5 mM dithiothreitol (DTT), 0.5 phenylmethylsulfonyl fluoride (PMSF), 2.0 μg/ml leupeptin and aprotinin] and placed on ice for 15 min with intermittent agitation. To recover the nuclear pellets and the cell lysates, samples were centrifuged at 3,300 *g* for 15 min. The supernatant was recovered for the determination of proteins by the bicinchoninic acid assay (BCA) method (Pierce Endogen, Rockford, IL). The pellet was resuspended in 50 μl of nuclear extraction buffer (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 0.5 mM DTT, 1 PMSF, 2.0 μg/ml leupeptin and aprotinin) and placed on ice for 30 min with agitation, after which samples were centrifuged at 20,000 *g* for 30 min. The supernatant was saved as nuclear extract and stored at −80°C until ready for use. A consensus NF-κB response element (5′-GTTGAGGGGACTTTCCCAGGGC-3′) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). This was end-labeled with [γ-^{32}P]ATP (New England Nuclear, Boston, MA) with T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Binding of nuclear proteins to the labeled oligonucleotide probe was done by incubating 15 μg nuclear proteins with 50,000 cpm of labeled probe for 30 min at room temperature in a binding buffer [2 mM HEPES, 50 mM KCl, 2 mM EDTA, 10% glycerol and 1% BSA (wt/vol)] in the presence of 2 μg poly(dI-dC) (Amersham, Piscataway, NJ) in a final reaction volume of 20 μl. For supershift assays, 2 μg of anti-p65 monoclonal antibody (Chemicon, Tamecula, CA) were incubated per reaction at room temperature for 30 min before the addition of the labeled nucleotides. The protein-DNA complexes were resolved on a 5% native polyacrylamide gel before autoradiography.

RNase protection assays. Total RNA was extracted from adipocytes using Trizol (Invitrogen, Carlsbard, CA) according to the manufacturer's instructions. The integrity of the total RNA was verified on 0.8% agarose gels before use. Cytokine expression was determined using a pig-specific multiprobe RNase Protection System (Pharmingen, San Diego, CA). Generally, 50 μg total RNA was incubated at 56°C with [^{32}P]UTP-labeled antisense RNA. Protected bands obtained after RNase digestion were resolved on a 5% denaturing acrylamide gel. Autoradiographs were prepared from dried gels, and signal intensity was quantified using a Digital Science Imaging System (V. 2.0.1, Kodak, New Haven, CT).

Western blotting. Adipocytes were lysed in a buffer (25 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 5 mM EDTA, 5 mM DTT, 2 mM PMSF, 10 μg/ml pepstatin A and leupeptin, 1 mM Na_2VO_3) and centrifuged at 14,000 rpm for 10 min for the recovery of cell lysate. The protein concentration was determined using the BCA method noted previously. Proteins were denatured at 95°C in loading buffer and separated on a 12.5% SDS-polyacrylamide gel. After transfer, the membranes were blotted with anti-phospho p44/42 MAPK (Cell Signaling, Beverly, MA) for 4 h at room temperature, and specific bands were detected with an alkaline phosphatase-conjugated secondary antibody (Upstate, Lake Placid, NY). The visualized bands were imaged as described for RNase protection assays.

Media ELISA for TNF-α and IL-6. Media concentration of TNF-α was determined with a porcine TNF-α ELISA kit (Biosource, Camarillo, CA) with a sensitivity of 6 pg/ml and a range of 15.6–1,000 pg/ml. All tests were done according to the manufacturer's protocols. Media IL-6 was determined with a porcine specific ELISA kit (R&D Systems, Minneapolis, MN) with a limit of detection of 10 pg/ml.

Statistical analyses. All data were checked for normality before being analyzed using the mixed-model analysis of a split-plot design. The fixed effect was the treatment, and the random effect was the replicate. The main effects (treatment and replicate) were tested against the treatment × replicate interaction. When protected by a significant *F*-test, mean separation was accomplished using the least-squares mean separation (pdiff) procedure (24).

RESULTS

LPS induces NF-κB nuclear translocation in pig adipocytes. Using electrophoretic mobility shift assays as a qualitative assessment of LPS-induced NF-κB translocation, we performed dose and duration experiments to confirm that NF-κB was activated in adipocytes incubated with LPS. As shown in Fig. 1*A*, there was a marked induction of NF-κB translocation to the nucleus of adipocytes incubated with LPS. The identity of the nuclear factor was confirmed by a supershift assay in which the mobility of the complex was retarded by preincubation with an antibody to the p65 subunit. Dose and time titration assays indicated that the NF-κB response to LPS was dose dependent over the range of 0.01–100 μg/ml of LPS (Fig. 1*B*) and transient with a peak response to a single dose occurring at ~2 h (Fig. 1, *C* and *D*).

Induction of IL-6 expression and secretion of IL-6 and TNF-α by LPS. We anticipated that the LPS-induced NF-κB translocation would result in the induction of cytokine expres-

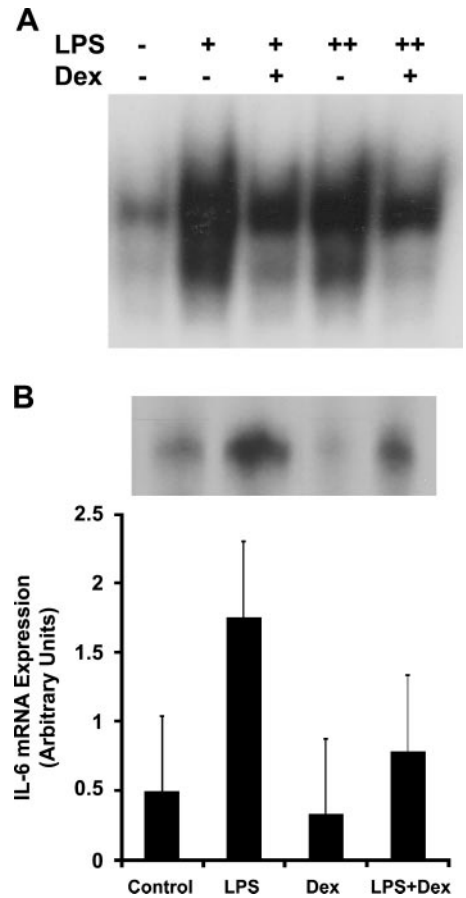

Fig. 3. Inhibition of LPS-induced NF-κB activation and IL-6 gene expression by dexamethasone (Dex). *A*: pretreatment of adipocytes for 2 h with dexamethasone (100 nM) before LPS treatment (+, 10 μg/ml; ++, 100 μg/ml) for an additional 2 h reduces the translocation of NF-κB caused by LPS. The experiment was repeated a minimum of 3 times with adipocytes from different pigs with similar results obtained. *B*: adipocytes were pretreated for 2 h with 100 nM dexamethasone before being treated for 3 h with LPS (10 μg/ml). Bars represent means ± SE from experiments performed with adipocytes from 3 different pigs.

sion in the stimulated adipocytes. Using a pig-specific multiprobe nuclease protection assay kit, we profiled the expression of several cytokines. As shown in Fig. 2, *A* and *B*, LPS induced the expression of IL-6, but we were unable to detect the mRNA for TNF-α, even after 3 h of exposure. Consequently, we assayed the medium for secreted IL-6 and TNF-α. There was a significant ($P < 0.05$) accumulation of IL-6 after 3 h (Fig. 2*C*) and a measurable increase in media TNF-α ($P < 0.09$, Fig. 2*D*) attributable to LPS after 8 h of incubation (Fig. 2*B*).

Regulation of LPS signaling in pig adipocytes by glucocorticoids. The anti-inflammatory properties of the synthetic glucocorticoid dexamethasone have been demonstrated both in vivo and in vitro in multiple cell types. However, direct evidence for the regulation of NF-κB by glucocorticoids in adipocytes is lacking. Accordingly, we determined whether dexamethasone would antagonize NF-κB translocation or the induction of cytokine expression by LPS in pig adipocytes. As shown in Fig. 3, *A* and *B*, respectively, dexamethasone attenuates LPS-induced NF-κB translocation in pig adipocytes and also had a tendency to decrease ($P < 0.09$) the induction of IL-6 expression by LPS.

Regulation of LPS signaling in pig adipocytes by selected kinases and G_i. In addition to NF-κB, the *Tlr-4* receptor is coupled to a number of other signal transducers, including several kinases and the inhibitory G protein G_i. To determine whether activation of the p44/42 MAP kinase pathway is an essential component of the induction of IL-6 expression by LPS, we tested the effect of the MEK inhibitor U-0126 on IL-6 expression. This inhibitor completely abolished the effect of LPS (Fig. 4*A*). Furthermore, Western blot analysis with antibody specific for the phosphorylated form of the p44/42 MAP kinase indicated that incubating adipocytes with LPS resulted in activation (phosphorylation) of this kinase (Fig. 4*B*), and the inhibitor blocked this activation by LPS (Fig. 4*C*). These changes were independent of changes in total p44/42 protein (not shown). However, the mechanism of MEK action may not involve NF-κB, as two selective inhibitors of MEK (PD-98059 and U-0126) failed to disrupt NF-κB translocation (data not

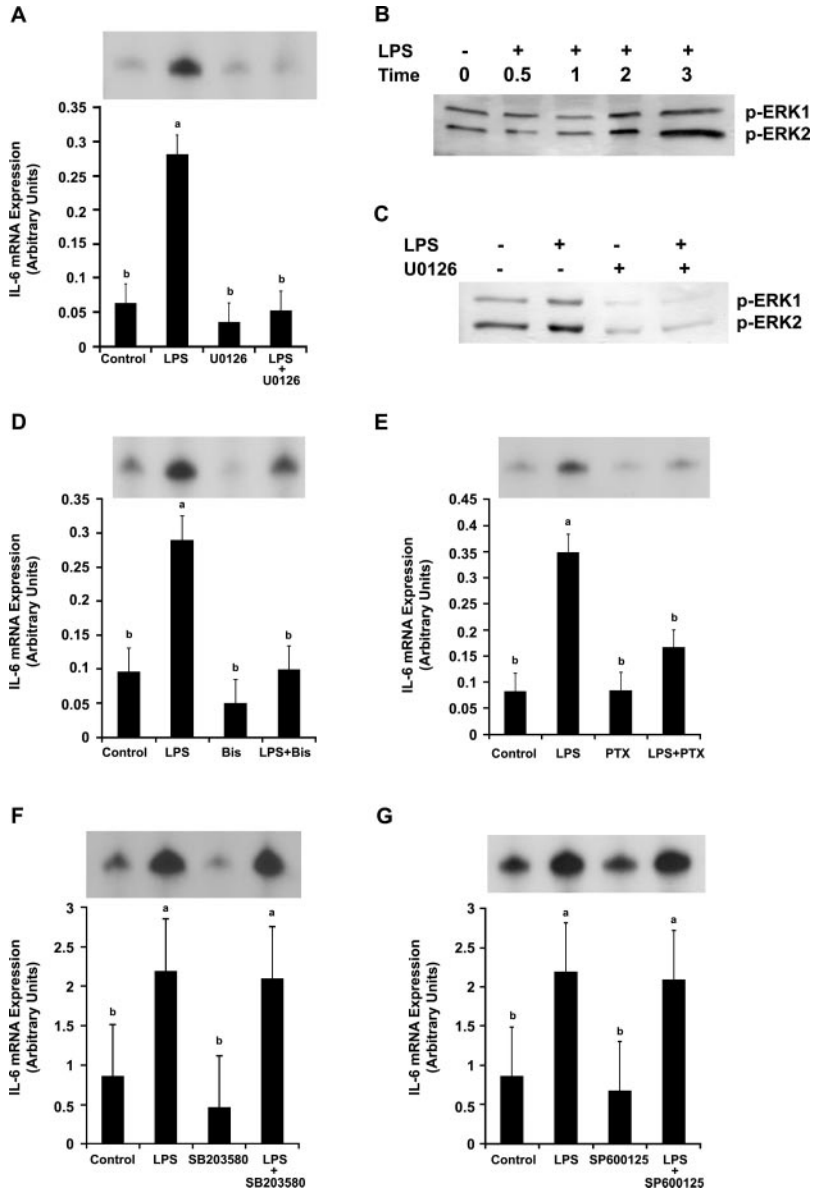

Fig. 4. Regulation of the induction of IL-6 expression in adipocytes by inhibitors of MEK (U-0126), p38 mitogen-activated protein kinase (SB-203580), c-Jun amino-terminal kinase (JNK, SP-600125), protein kinase C [bisindolylmaleimide HCl (Bis)], $G\alpha_i$ [pertussis toxin (PTX)]. Adipocytes were pretreated for 2 h with the respective inhibitors before the addition of LPS (10 μg/ml) for an additional 3 h. All experiments were replicated a minimum of 3 times with adipocytes isolated from different pigs. *A*: MEK inhibition with U-0126 inhibits the induction of IL-6 expression by LPS. Bars represent means ± SE. Means with different lowercase letters differ at $P < 0.01$. *B*: LPS induces phosphorylation of ERK1/2. pERK1/2, phospho-ERK1/2. *C*: U-0126 inhibits ERK1/2 phosphorylation. Adipocytes were pretreated with U-0126 for 2 h before LPS (10 μg/ml) treatment for an additional 3 h after which Western blotting was performed on the cell lysate. *D*: pretreatment of adipocytes with bisindolylmaleimide HCl for 2 h before LPS (10 μg/ml) treatment suppresses IL-6 induction. Means with different lowercase letters are different at $P < 0.01$. *E*: inhibition of $G\alpha_i$ with pertussis toxin inhibits the induction of IL-6 expression by LPS. Means with different lowercase letters differ at $P < 0.05$. *F*: SB-203580 had no effect on LPS-induced IL-6 expression. Means with different lowercase letters differ at $P < 0.05$. *G*: JNK inhibition with SP-600125 had no effect on the regulation of IL-6 expression by LPS. Means with different lowercase letters differ ($P < 0.01$).

shown) at the concentrations that blocked IL-6 expression and phosphorylation of p44/42 MAP kinase. As with MEK, incubating stimulated adipocytes with the PKC inhibitor bisindolylmaleimide abolished ($P < 0.01$) the induction of IL-6 by LPS (Fig. 4*D*). Disabling G_i by ADP-ribosylation with pertussis toxin also precluded the induction of IL-6 expression by LPS (Fig. 4*E*). However, inhibition of p38 and C-JNK with SB-203580 and SP-600125, respectively, did not diminish NF-κB translocation or IL-6 expression (Fig. 4, *F* and *G*).

Regulation of IL-15 expression: effect of LPS, IFN-γ, and selected kinase inhibitors. As indicated in Fig. 5*A*, a total of three experiments with cells isolated from different pigs show that primary pig adipocytes express the mRNA for IL-15 under basal conditions. Although unresponsive to LPS, adipocyte expression of this cytokine is markedly induced by IFN-γ. The induction of IL-15 by IFN-γ is significant ($P < 0.03$) by 2 h and is increased further by 3 h ($P < 0.01$, Fig. 5*B*). Whereas the ability of IFN-γ to induce IL-15 expression is not altered by the MEK inhibitor U-0126, it is highly sensitive to inhibition of PKC; the induction of IL-15 by IFN-γ was blocked completely ($P < 0.01$) by incubation with bisindolylmaleimide HCl (Fig. 5*C*).

DISCUSSION

The *Toll* receptors and the NF-κB transcription factor play crucial roles in the regulation of innate immunity in species ranging from *Drosophila* to humans (reviewed by Ref. 12). Previously, Lin et al. (17) showed that 3T3-L1 adipocytes respond directly to LPS with increased expression of IL-6 and an upregulation of Tlr-*2* receptor synthesis. In the present study, we have confirmed that the primary pig adipocyte also responds directly to LPS with an increase in the expression of IL-6 as was reported for the 3T3-L1 cell line. We also show that the increase in IL-6 expression is associated with increased secretion of the protein into the culture medium and that the NF-κB transcription factor is translocated to the nucleus of adipocytes stimulated with LPS. Furthermore, there was a tendency for these responses to be attenuated by dexamethasone, a classical anti-inflammatory agent. The NF-κB transcription factor is a major regulator of proinflammatory cytokine expression in macrophages stimulated with LPS (1, 2, 7). Thus our determination that LPS causes NF-κB translocation to the nucleus further substantiates the new view of the adipo-

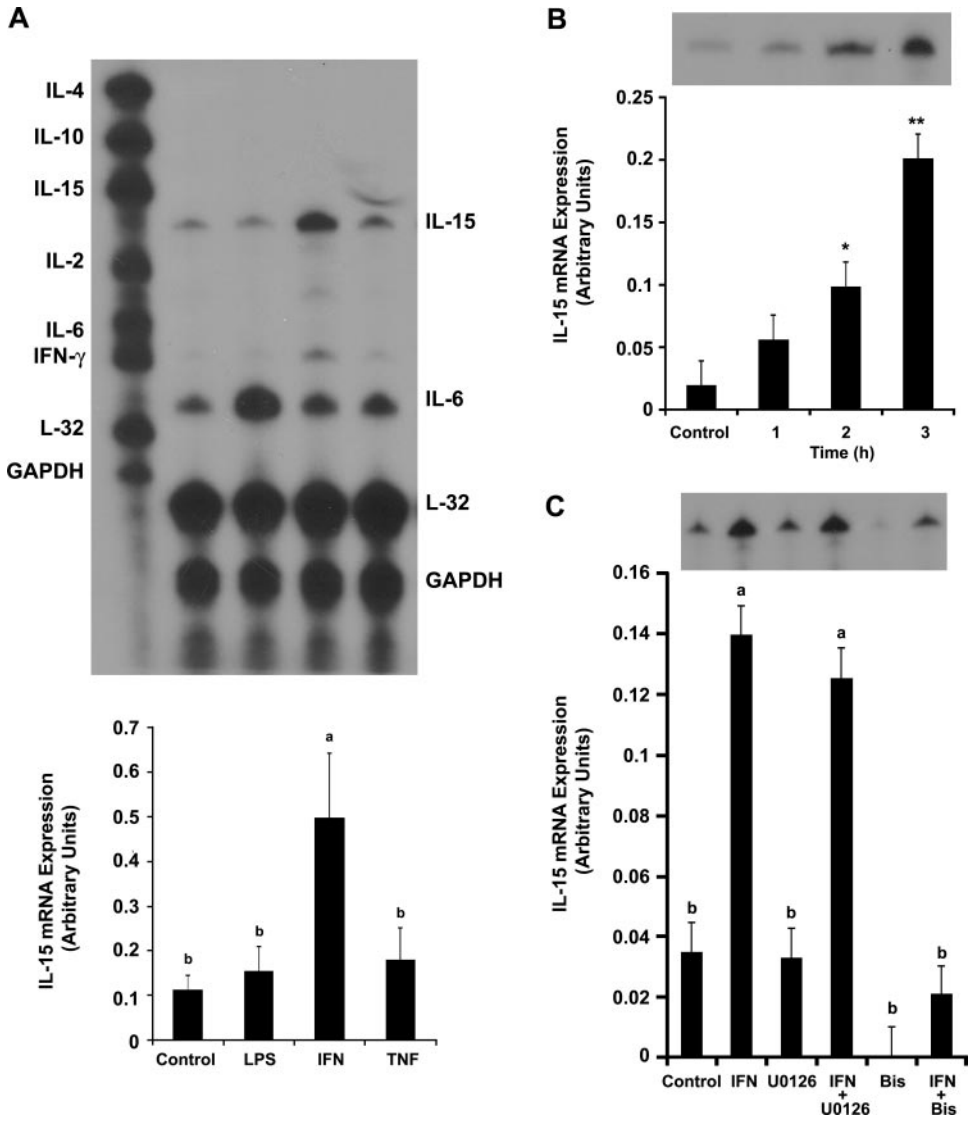

Fig. 5. Induction of IL-15 mRNA expression in adipocytes treated with LPS, IFN-γ, or TNF-α. *A*: adipocytes were treated with LPS (10 μg/ml), IFN-γ (50 ng/ml), or TNF-α (50 ng/ml) for 3 h. The experiment was replicated 5 times with adipocytes isolated from different pigs. Bars represent means ± SE. Means with different lowercase letters differ at $P < 0.01$. *B*: time course of induction of IL-15 mRNA by IFN-γ. Adipocytes were collected at *time 0* (control) or treated with IFN-γ (50 ng/ml) for 1, 2, or 3 h. Differences from control: $*P < 0.03$, $**P < 0.0001$. *C*: adipocytes were pretreated for 1 h with the MEK inhibitor U-0126 or the PKC inhibitor bisindolylmaleimide HCl before being treated with IFN-γ (50 ng/ml) for 3 h. The experiment was replicated 3 times with adipocytes isolated from different pigs. Bars represent means ± SE. Means with different lowercase letters differ at $P < 0.01$.

cyte as an active participant in the innate immune response. However, it is also noteworthy that we were unable to detect the expected induction of TNF-α expression in pig adipocytes by LPS and that 8 h was required to detect an LPS-induced increase in the media TNF-α concentration. The implication may be one of a notable species difference between the pig- and rodent-derived adipocytes, despite the clear similarities in other parts of the response pathway.

The importance of ERK1/2, PKC, and G_i to the LPS-induced expression of IL-6 in the adipocyte is quite apparent in that inhibition of their activity abolished the ability of LPS to induce IL-6 expression. In contrast, inhibition of the JNK and p38 MAP kinase activities was inconsequential and thus indicates that these kinases are not central to the induction of IL-6 expression by LPS. This is in marked contrast with recent findings in C2C12 myocytes, in which the induction of IL-6 requires JNK activity (10). The IL-6 promoter is regulated by multiple transcription factors, including CCAAT/enhancer binding protein, activator protein (AP)-1, cAMP response element-binding protein, and NF-κB (29). However, NF-κB typically acts as the terminal signal for the activation of the enhanceosome on the IL-6 promoter (29). In the present study, clear disruption of the LPS-induced IL-6 expression by inhibition of ERK1/2 did not correspond with a visible reduction in NF-κB translocation to the nucleus, based on the mobility shift assay. This seemingly indicates the importance of AP-1 or other components of the enhanceosome in the regulation of IL-6 expression. This concept is supported by the fact that blocking NF-κB activity by preventing degradation of IkBα with a proteosome inhibitor did not abrogate the induction of IL-6 in myocytes stimulated with LPS (10).

The inhibition of LPS-induced IL-6 expression by pertussis toxin indicates that the G_i is an important component of the regulation of IL-6 in activated adipocytes and is consistent with the conclusion by Solomon et al. (26) that a physical association of heterotrimeric G proteins with CD14 is essential for the response to LPS. In fact, the regulatory actions of the G_i may contribute to the activation of ERK1/2 by LPS (9), which was also critical to the induction of IL-6 expression. Likewise, in primary astrocyte cultures stimulated with LPS, Lee et al. (15) found that inhibition of PKC activity blocked the LPS-induced activation of ERK1/2 and matrix metalloproteinase-9 expression. This indicates the possibility that LPS induced a sequential activation of PKC and ERK1/2 and that the mechanism by which inhibition of PKC or G_i activity disrupted the induction of IL-6 expression by LPS may relate directly to the activation of ERK1/2.

The adipocyte has been well-documented as a significant source of inflammatory cytokines and other regulatory factors (reviewed by Ref. 6). However, to our knowledge, this is the first report of either constitutive or IFN-γ-inducible expression of IL-15 in adipocytes. This finding raises two important questions. First of all, it is of particular interest that LPS did not directly induce either IFN-γ or IL-15 expression in the adipocyte, although it does increase circulating IFN-γ concentrations in the pig in vivo (5). Thus it will be of considerable importance to determine whether IL-15 expression is increased in the adipocyte in vivo. Second, it will be important to determine whether the increase in IL-15 mRNA corresponds with an increase in secretion of the cytokine. IL-15 is in some respects proinflammatory in its actions, which include the induction and perpetuation of chronic inflammation (16) and the recruitment (8, 21) and survival (3) of memory T-cells. However, in rodent models, IL-15 also promotes muscle growth (4, 23) and decreases adipose mass (4). Thus it is conceivable that this cytokine alters metabolic pathways in the adipocyte and myofiber as a part of the physiological adaptation required during inflammation.

In conclusion, we have confirmed in primary pig adipocytes that LPS activates the NF-κB transcription factor and stimulates the expression and secretion of IL-6. We determined that ERK1/2, PKC, and G_i are critical components of the LPS signaling pathway, whereas the p38 MAPK and JNK are not. Herein, we also provide the first evidence of IL-15 expression in the adipocyte of any species and show clearly that IFN-γ and PKC are major regulators of IL-15 expression. Collectively, these findings are interpreted as additional evidence that the adipocyte plays a unique role in the innate immune response.

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