Interaction of CD44, an adhesion molecule, with its ligand, hyaluronan (HA), in monocytic cells plays a critical role in cell migration, inflammation, and immune responses. Most cell types express CD44 but do not bind HA. The biological functions of CD44 have been attributed to the generation of the functionally active, HA-adhesive form of this molecule. Although lipopolysaccharide (LPS) and cytokines induce HA-adhesive CD44, the molecular mechanism underlying this process remains unknown. In this study, we show that LPS-induced CD44-mediated HA (CD44-HA) binding in monocytes is regulated by endogenously produced tumor necrosis factor (TNF)-α and IL-10. Furthermore, p38 mitogen-activated protein kinase (MAPK) activation was required for LPS- and TNF-α-induced, but not IL-10-induced, CD44-HA-binding in normal monocytes. To dissect the signaling pathways regulating CD44-HA binding independently of cross-regulatory IL-10-mediated effects, IL-10-refractory promonocytic THP-1 cells were employed. LPS-induced CD44-HA binding in THP-1 cells was regulated by endogenously produced TNF-α. Our results also suggest that lysosomal sialidase activation may be required for the acquisition of the HA-binding form of CD44 in LPS- and TNF-α-stimulated monocytic cells. Studies conducted to understand the role of MAPKs in the induction of sialidase activity revealed that LPS-induced sialidase activity was dependent on p42/44 MAPK-mediated TNF-α production. Blocking TNF-α production by PD98059, a p42/44 inhibitor, significantly reduced the LPS-induced sialidase activity and CD44-HA binding. Subsequently, TNF-α-mediated p38 MAPK activation induced sialidase activity and CD44-HA binding. Taken together, our results suggest that TNF-α-induced p38 MAPK activation may regulate the induction of functionally active HA-binding form of CD44 by activating sialidase in LPS-stimulated human monocytic cells.

CD44, an adhesion molecule, comprises a family of 85–200-kDa transmembrane glycoproteins that are widely expressed in a variety of cell types (1). CD44 binds to high endothelial venules and to the extracellular matrix via its interaction with its principle ligand, hyaluronan (HA) (1–3). CD44-HA interactions have been implicated mainly in cell-cell and cell-matrix adhesion and hence play a key role in a variety of physiological processes, including cell migration, lymphocyte homing, cell activation, and hemopoiesis, as well as in disease processes such as arthritis, inflammation, and tumor metastasis (1, 3, 10–14).

The ability of CD44 to bind HA is a tightly regulated process and is dependent on cell type, state of cell activation, and differentiation (1, 14–17). Although most cells express some form of CD44, not all cells constitutively bind HA (17, 18). Acquisition of the HA-binding ability of CD44 thus plays a vital role in determining CD44-mediated biological effects. The HA-binding capacity of CD44 has been suggested to be influenced by multiple factors that include structural variations in the CD44 extracellular domain, oligomerization of CD44 on the cell membrane, and phosphorylation of its cytoplasmic tail (1, 10–12, 14, 19, 20). It is also known that alterations in the N- and O-linked glycosylation patterns of CD44 play a vital role in the regulation of its binding to HA (13, 19–21). Recently, an inducible sialidase was implicated in CD44-HA binding of lipopolysaccharide (LPS)-stimulated human monocytic cells (22).

The lipopolysaccharide component of endotoxin, derived from Gram-negative bacterial cell walls, induces inflammatory responses that contribute to the pathogenesis of sepsis, inflammation, and a number of autoimmune diseases including rheumatoid arthritis. It is well established that mononuclear phagocytes play a major role in the pathogenesis of LPS-induced syndromes. Peripheral blood monocytes express abundant cell surface CD44 yet do not bind HA (23, 24). It was recently reported that stimulation of monocytes with LPS up-regulated CD44-mediated HA-binding (23, 24). TNF-α, a proinflammatory cytokine, was shown to be an important positive regulator

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.
of LPS-induced CD44-HA binding in these cells (24). Furthermore, ligation of CD44 with HA has been shown to induce a number of proinflammatory cytokines including TNF-α (25, 26). Therefore, the acquisition of HA binding capacity by CD44 expressed on monocytic cells could be critical for their participation in inflammatory responses. Modulation of CD44-HA binding in monocytic cells by endotoxins and inflammatory cytokines may thus have profound effects on the migration of monocytes to sites of inflammation and on the development of immune responses. Hence, understanding the signaling pathways governing the regulation of CD44 expression and the synthesis of the HA-adhesive form of CD44 may lead to the development of strategies for the treatment of autoimmune diseases and cancer.

There is very little information available regarding the molecular mechanisms involved in the regulation of HA-adhesive, functionally active CD44 expression (27). We have recently demonstrated the involvement of c-Jun-N-terminal kinase (JNK) in the regulation of LPS-induced CD44 expression in human monocytic cells (28). In this study, we examined the mitogen-activated protein kinase (MAPK) transduction pathways involved in the synthesis of the HA-adhesive, functionally active form of CD44 in LPS-stimulated normal human monocytes and in promonocytic THP-1 cells. MAPKs are serine-threonine protein kinases that include p38, p42/44 extracellular signal-regulated kinases (ERKs), and JNK (29, 30). MAPKs play a key role in cellular responses such as proliferation, differentiation, and apoptosis (29–31). These three MAPKs are involved in parallel signaling cascades activated by distinct and sometimes overlapping sets of stimuli. In general, ERKs are activated by growth factors, whereas the p38 and JNK are activated by stress stimuli (29, 30). LPS and TNF-α have been shown to induce the expression of several cellular genes via activation of all three classes of MAPKs (29, 32–35).

In this study, we show that LPS-induced CD44-HA binding in normal monocytes is regulated by endogenously produced TNF-α and IL-10. Studies designed to delineate the role of MAPKs revealed that p38 activation was required for LPS- and TNF-α-induced expression of the HA-adhesive CD44. In contrast, IL-10-induced CD44-HA binding was not mediated through either either p38 or p42/44 activation. To further dissect the TNF-α- and LPS-induced signaling pathways independent of IL-10-mediated effects, we utilized IL-10 refractory THP-1 cells as a model system. We show that TNF-α production in LPS-stimulated THP-1 cells involves p42/44 MAPK activation, and endogenously produced TNF-α is required for the induction of CD44-mediated HA binding. Our results also show that sialidase activation is required for the acquisition of HA binding capacity by CD44 in LPS- and TNF-α-stimulated monocytic cells. These results prompted us to investigate a role for p38 and p42/44 in LPS- and TNF-α-induced sialidase activity resulting in the synthesis of the HA-binding form of CD44. LPS-induced sialidase activity was dependent on p42/44 MAPK-mediated TNF-α production. Blocking TNF-α production by PD98059, a p42/44 inhibitor, significantly reduced the LPS-induced sialidase activity and CD44-HA binding. Furthermore, TNF-α-induced sialidase activity and CD44-HA binding was found to be regulated by p38 MAPK activation. Taken together, these results suggest a key role for TNF-α in regulating the synthesis of HA-adhesive CD44 by inducing sialidase activity through p38 MAPK activation in LPS-stimulated monocytic cells.

**EXPERIMENTAL PROCEDURES**

**Cell Lines, Cell Culture, and Reagents**—THP-1, a promonocytic cell line derived from a human acute lymphocytic leukemia patient, was obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in Iscove’s Dulbecco’s medium (Sigma) supplemented with 10% fetal bovine serum (Invitrogen), 100 units/ml penicillin, 100 μg/ml gentamicin, 10 mM HEPES, and 2 mM glutamine. PD98059 (Calbiochem), an inhibitor of MEK1 kinase, selectively blocks the activity of ERK MAPK and has no effect on the activity of other serine-threonine protein kinases, including Raf-1, p38, and JNK MAPKs, or protein kinase C and protein kinase A (36, 37). The pyridinyl imidazole SB202190 (Calbiochem), a potent inhibitor of p38 MAPK, has no significant effect on the activity of the ERK or JNK MAPK subgroups (36, 37). SB202474, an inactive analog of SB202190, was also purchased from Calbiochem. LPS derived from Escherichia coli 0111:B4 (Sigma), recombinant human IL-10 (R&D Systems, Minneapolis, MN), recombinant human TNF-α (BioSource, Montreal, Canada), and human IL-10Ra and human TNF-αR1 antibodies (R&D Systems) capable of neutralizing the biological activities of IL-10 and TNF-α, respectively, were obtained. Hyaluronan preparation (Calbiochem) was more than 97% pure. Sialidase from Arthrobacter ureafaciens, sialidase inhibitor, 2,3-dehydro-2-deoxy-N-acetylmuramic acid (2-NeuAc), and all other chemicals used for Western blotting were purchased from Sigma. The sialidase inhibitor, 2-NeuAc, is a transition state analogue of sialic acid and is a potent inhibitor of viral, bacterial, and mammalian sialidases (38).

**Isolation of Monocytes from PBMCs**—PBMCs were isolated from the blood of healthy adult volunteers following approval of the protocol by the Ethics Review Committee of the Children’s Hospital of Eastern Ontario (Ottawa, Canada). PBMCs were isolated by density gradient centrifugation over Ficoll-Hypaque (Amersham Biosciences) as previously described (29, 35). Purified, nonactivated monocytes were obtained by a negative selection procedure involving depletion of T cells and B cells using magnetic polystyrene M-450 Dynabeads (DynaL, Oslo, Norway) coated with antibodies specific for CD2 (T cells) or CD19 (B cells), as described earlier (28, 35). Briefly, PBMCs (10 x 10⁶/ml) were resuspended with CD2 and CD19 Dynabeads and were incubated for 30 min on ice with constant mixing. Cells were incubated at 37 °C for 2 h, following which nonadherent cells were removed. The adherent mononuclear cells obtained contained fewer than 1% CD2+ T cells and CD19+ B cells as determined by flow cytometry.

**Measurement of IL-10 and TNF-α Production in Culture Supernatants by Enzyme-linked Immunosorbent Assay**—To determine the effects of p38 and p42/44 MAPK inhibitors on CD44-mediated HA binding and IL-10 and TNF-α production, purified human monocytes (1 x 10⁶/ml) and THP-1 cells (0.5 x 10⁶/ml) were stimulated for 24 h with LPS (1 μg/ml in the presence or the absence of MAPK inhibitors. Cells were analyzed for CD44-mediated HA binding, and the supernatants were analyzed for IL-10 and TNF-α production by enzyme-linked immunosorbent assay. IL-10 and TNF-α were measured by employing two different monoclonal antibodies (mAbs) recognizing distinct epitopes as described earlier (28, 35). Briefly, the following primary antibodies were used for coating the plates (anti-IL-10 mAb 18551D from BD Pharmingen (Mississauga, Canada); final concentration of 5 μg/ml; anti-TNF-α mAb AHC3712 from BIOSOURCE; final concentration of 5 μg/ml). IL-10 or TNF-α were detected using a second biotinylated mAb (anti-IL-10 antibody 18562D from Pharmingen, final concentration of 4 μg/ml; anti-TNF-α antibody AHC3419 from BIOSOURCE, final concentration of 4 μg/ml). Streptavidin peroxidase was used at a final concentration of 1:1000 (Jackson ImmunoResearch, West Grove, PA). The color reaction was developed by 0-phenylenediamine (Sigma) and hydrogen peroxide, and the absorbance was read at 450 nm. IL-10 (R&D Systems) and TNF-α (BIOSOURCE) were used as standards. The level of sensitivity for both IL-10 and TNF-α production was 16 pg/ml.

**HA Adhesion Assay**—CD44-mediated binding assays were performed as described previously (17, 39). Briefly, stimulated cells (2 x 10⁶/ml) were resuspended in Iscove’s Dulbecco’s medium containing bovine serum and were pulsed for 1.5 h with ³⁵Cr (sodium chromate; Amersham Biosciences) at a concentration of 300 μCi/10⁶ cells. After three washes, cells were aliquoted at a concentration of 2 x 10⁶ cells/ml into 96-well microtiter plates (NUNC, Immunomodules, Roskilde, Denmark), which had been coated overnight with 1 mg/ml of either umbilical cord hyaluronan (Sigma) or, as a negative control, chondroitin sulfate C (Calbiochem). Cells were incubated for 1.5 h at 37 °C and were washed six times with warm Iscove’s Dulbecco’s medium, 10% fetal bovine serum to remove nonadherent cells. The adherent cells were lysed with 1 N HCl, and radioactivity was measured by scintillation counting using a Microbeta counter (Wallac, Turku, Finland). The percentage of adherent cells was determined as follows:

\[
\text{% adherent cells (\% cpm total input)} = \frac{\text{cpm adherent} - \text{spontaneous release cpm}}{\text{Total input cpm} - \text{spontaneous release cpm}} \times 100 \quad (\text{Eq. 1})
\]
Western Blot Analysis—Phosphorylation of p38 or p42/44 ERK MAPKs was determined by Western blot analysis as previously described (28, 35, 39). Briefly, cells were stimulated at 37 °C for 0–15 min with either LPS, IL-10, or TNF-α. Cells pellets were lysed, and the protein concentration was determined by using the Bio-Rad protein determination assay (Bio-Rad). Total cell proteins were subjected to 8% polyacrylamide SDS gel electrophoresis followed by transfer onto polyvinylidene difluoride membranes (Bio-Rad). The membranes were probed with either rabbit anti-phospho-p38 mAb (New England Biolabs, Mississauga, Canada) or mouse anti-phospho-p42/44 mAb (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), followed by horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse polyclonal antibodies (Bio-Rad). The membranes were incubated in a stripping buffer (62.5 mM Tris-HCl, pH 6.7, 100 mM 2-β-mercaptoethanol, 2% SDS, and 0.7 mM dithiothreitol) for 30 min at 50 °C with gentle agitation. The membranes were washed with TBST buffer (150 mM Tris-HCl, 1 mM NaCl, and 1% Tween 20) seven times followed by reprobing with rabbit polyclonal antibodies specific for the unphosphorylated forms of either p38 or p42 MAPKs (Santa Cruz Biotechnology). All immunoblots were visualized by ECL (Amersham Biosciences).

Determination of Sialidase Activity—Measurement of lysosomal and plasma membrane sialidase activity was performed as described (22). Cells (15 × 10^6) were washed with phosphate-buffered saline and resuspended in ice-cold buffer containing 0.25 M sucrose, 1 mM EDTA, and 0.2 mM phenylmethylsulfonyl fluoride. The cell suspension was sonicated on ice for 10 s on a low setting (7% amplitude) (VibraCellTM; Sonics and Materials Inc., Newtown, CT) followed by centrifugation at 25,000 × g for 15 min at 4 °C. The resulting supernatant was used to determine the lysosomal sialidase activity. Protein quantification of the supernatant was performed using the Bio-Rad protein determination kit as described above. For the determination of lysosomal sialidase activity, protein quantification of the supernatant was performed using the Bio-Rad protein determination kit as described above. For the determination of lysosomal sialidase activity, the total protein was mixed with 40 nmol of 4-methylumbelliferyl-α-N-acetyl-α-neuraminic acid (Sigma), the lysosomal sialidase-specific substrate, 10 μmol sodium acetate buffer, pH 4.6, and 200 μg of bovine serum albumin in a total volume of 200 μL. The sialidase reaction was allowed to proceed for 1 h at 37 °C and was terminated by the addition of 0.25 g glycine NaOH, pH 10.4. Released 4-methylumbelliferyl α-N-acetyl-α-neuraminic acid was measured fluorometrically (PerkinElmer Life Sciences) in a sodium carbonate buffer at an excitation wavelength of 365 nm and emission wavelength of 448 nm as described previously (40). One unit of sialidase activity was defined as the amount of enzyme that catalyzed the release of 1 nmol of sialic acid/h. The measurement of lysosomal sialidase activity was optimized using various concentrations of total protein (25, 50, 100, 150, and 200 μg). The maximal sialidase activity was detected when 200 μg of total cellular proteins was used. Furthermore, the optimal incubation time for sialidase activity was determined to be 1 h.

Lysosomal sialidase activity was determined in THP-1 cells stimulated with LPS or TNF-α for varying periods of time. The sialidase activity for these experiments was expressed as a measure of fluorescence intensity. To determine the involvement of MAPKs in the LPS- or TNF-α-induced lysosomal sialidase activity, cells were pretreated with these inhibitors for 2 h followed by stimulation of monocytes with LPS, TNF-α, or IL-10. LPS and TNF-α enhanced CD44-HA binding (Fig. 1). Furthermore, stimulation of monocytes with exogenous TNF-α and IL-10 enhanced CD44-HA binding (Fig. 1) in a dose-dependent manner (data not shown). To determine whether the binding of cells to HA was mediated through CD44, monocytic cells stimulated with either LPS, TNF-α, or IL-10 were cultured with anti-CD44 antibodies prior to incubation with HA. Treatment of monocytic cells with anti-CD44 antibodies inhibited LPS-, TNF-α-, and IL-10-induced HA binding (Fig. 1).

LPS- and TNF-α-induced CD44-mediated HA Binding Involves the Activation of p38 MAPK—The MAPKs play a major role in LPS- and cytokine-mediated induction of several cell surface receptors including CD44 (28–30, 32–35, 43). It was, therefore, of interest to identify the members of the MAPK family that are involved in LPS-, IL-10-, and TNF-α-induced regulation of CD44-HA binding in monocytic cells. We first investigated the phosphorylation of p38 and p42/44 MAPKs by examining their activation following stimulation of monocytes either with LPS, TNF-α, or IL-10. LPS and TNF-α induced the phosphorylation of both p38 and p42/44 ERKs (Fig. 2, A and B). In contrast, IL-10 enhanced the phosphorylation of p42/44 ERKs but had no effect on p38 phosphorylation (Fig. 2C). The role of p38 and p42/44 ERKs has been studied through the use of specific kinase inhibitors: SB202190 for p38-mediated and PD98059 for p42/44-mediated signaling (37). To confirm that in our system SB202190 and PD98059 inhibited the phosphorylation of p38 and ERKs, respectively, freshly isolated monocytes were pretreated with these inhibitors for 2 h followed by stimulation with either LPS, TNF-α, or IL-10 for 10 min. Prior treatment of monocytes with SB202190 or PD98059 resulted in the inhibition of both LPS- and TNF-α-induced p38 and p42/44

containing 5% (v/v) 12% HC1) was added to the reaction mixture, mixed, and centrifuged to separate the aqueous and organic phases. The color reaction in the butanol layer was measured by reading the absorbance in a spectrophotometer at a wavelength of 549 nm. Membrane sialidase activity was expressed as ng of sialic acid released/200 μg of total protein. Purified sialic acid (Sigma) at concentrations ranging from 0 to 40 μg was used to generate a standard curve using the Aminoff method. The membrane (lysosomal) sialidase activity was determined by comparing the absorbances obtained from the cell samples with the absorbances measured from the standard curve.

Analysis of Soluble and Membrane-associated Lysosomal Sialidase Activity—To determine whether the lysosomal sialidase was localized in the cell membrane or in the soluble cytosol fraction, the cells were stimulated for 16 h. The cell pellet was washed and treated with 1% digitonin for 45 min on ice followed by centrifugation at 14,000 × g for 20 min at 4 °C. The supernatant constituted the soluble cytosol fraction, whereas the pellet represented the membrane fractions. The membrane fraction was solubilized in a nonionic detergent, 1% Nonidet P-40. The lysosomal sialidase activity was determined in both the cytosol and membrane fractions as per the methods described above.

Statistical Analysis—Means were compared using the two-tailed Student’s t test. Results are expressed as means ± S.E.
ERK phosphorylation, respectively (Fig. 2, A and B). Similarly, pretreatment of monocytes with PD98059 prior to IL-10 stimulation inhibited p42/44 phosphorylation in a dose-dependent manner (Fig. 2C).

To determine the role of p38 and p42/44 MAPKs in LPS-induced CD44-HA-binding, monocytes were pretreated with SB202190 or PD98059 for 2 h before LPS stimulation for 24 h. The cells were analyzed for CD44-mediated HA-binding by the HA adhesion assay. CD44-HA binding was significantly inhibited at doses as low as 5 μM (Fig. 3). Treatment of monocytes with SB202474, an inactive analogue of SB202190, for 2 h prior to LPS stimulation did not influence CD44-HA binding (data not shown), thus confirming the specificity of SB202190. PD98059 did not inhibit CD44 binding at doses up to 50 μM (Fig. 3), indicating that the p42/44 pathway is not involved in the regulation of LPS-induced CD44-HA binding.

To determine whether the inhibition of CD44-HA binding observed following treatment with SB202190 of LPS-stimulated monocytes was due to the inhibition of endogenous IL-10 and/or TNF-α expression, we evaluated the effects of MAPK inhibitors on IL-10 and TNF-α production. SB202190, but not PD98059, inhibited LPS-induced IL-10 as well as TNF-α production in a dose-dependent manner (Table I). The same cells were analyzed for HA binding as well as for IL-10 and TNF-α production. These results suggest that the inhibition of LPS-induced CD44-mediated HA binding by SB202190 may be, at least in part, due to the decreased endogenous production of TNF-α and/or IL-10.

It is likely that IL-10 and/or TNF-α produced following LPS stimulation of monocytes may up-regulate CD44-HA-binding by activating either p38 or p42/44 MAPKs. To determine whether p38 and p42/44 MAPKs play a role in TNF-α-induced CD44-HA binding, monocytes were pretreated with either p38 or p42/44 inhibitors for 2 h followed by TNF-α stimulation. Similar to the results obtained for LPS-induced CD44-HA binding (Fig. 3A), neither SB202474 (data not shown) nor
PD98059 influenced TNF-α-induced CD44-HA binding. In contrast, SB202190 significantly inhibited CD44-HA binding at doses as low as 5 μM, and complete inhibition was observed at doses of 25 μM (Fig. 3B). Furthermore, PD98059 did not inhibit IL-10-induced CD44-mediated HA binding (Fig. 3C). These results suggest that p38 may play a role in both LPS- and TNF-α-induced CD44-mediated HA-binding.

**LPS-induced CD44-HA Binding in THP-1 Cells Is Regulated by Endogenously Produced TNF-α**—LPS-induced CD44-HA-binding in normal monocytes is a complex process that is regulated by the interaction of LPS with its CD14-Toll-like receptor complex as well as by the interaction between IL-10 and TNF-α with their corresponding receptors (44–46). Because of the inherent endogenous production of IL-10 in LPS-stimulated monocytes, the role of p38 and p42/44 MAPK in LPS- and TNF-α-induced CD44-HA-binding could not be defined. To investigate the role of MAPKs in LPS-induced CD44-HA binding independent of endogenous IL-10, we employed IL-10-refractory THP-1 cells as a model system. THP-1 cells responded in a similar manner as monocytes with respect to CD44-HA binding following stimulation with either LPS or TNF-α (Fig. 4). Furthermore, treatment of LPS-stimulated THP-1 cells with anti-

*Fig. 3. The p38 MAPK inhibitor modulates LPS- and TNF-α-induced CD44-mediated HA binding in purified normal monocytes. Cells (0.5 x 10⁶/ml) were pretreated with either SB202190 or PD98059 at varying concentrations ranging from 0 to 50 μM for 2 h prior to LPS (1 μg/ml) (A), TNF-α (10 ng/ml) (B), or IL-10 (10 ng/ml) (C) stimulation for 24 h. CD44-mediated HA binding was analyzed by the HA adhesion assay. Percentages of adherent cells are presented as the mean of triplicate samples ± S.D. The results shown are representative of three experiments.*

**TABLE I**

| Cytokine | Treatment | Cytokine production (pg/ml) following LPS stimulation in the presence of MAPK inhibitors |
|----------|-----------|----------------------------------------------------------------------------------|
|          |           | 0 μM  | 0.625 μM | 1.25 μM | 2.5 μM | 5 μM | 10 μM | 25 μM |
| IL-10    | LPS + SB202190 | 218.5 ± 13.4 | 75.5 ± 7.8 | 74.0 ± 2.8 | 83.2 ± 4.2 | 64.1 ± 1.4 | 62.6 ± 2.8 | 41.4 ± 5.7 |
|          | LPS + PD98059 | 386.2 ± 11.3 | 348.5 ± 65.8 | 397.5 ± 58.7 | 401.5 ± 14.8 | 435.0 ± 12.7 | 438.5 ± 67.9 | 363.5 ± 54.4 |
| TNF-α    | LPS + SB202190 | 244.5 ± 9.1 | 76.3 ± 5.1 | 95.7 ± 10.6 | 67.3 ± 6.8 | 72.4 ± 11.8 | 66.1 ± 15.6 | 64.6 ± 7.1 |
|          | LPS + PD98059 | 467 ± 15.6 | 681.5 ± 37.4 | 662.5 ± 55.9 | 556.5 ± 68.8 | 538.5 ± 4.9 | 485 ± 80.6 | 475.5 ± 19.1 |

* p ≤ 0.05. Unstimulated cells produced less than 16 pg/ml of IL-10 or TNF-α in the culture supernatants.
TNF-αR1 antibodies inhibited CD44-HA binding, suggesting that LPS-induced CD44-HA binding is regulated by endogenously produced TNF-α (Fig. 4). In addition, treatment of THP-1 cells with anti-CD44 antibodies inhibited LPS- and TNF-α-induced HA binding (Fig. 4).

**LPS- and TNF-α-induced CD44-HA Binding in THP-1 Cells Involves Differential Activation of p38 and p42/44 MAPKs**—To examine the role of p38 and p42/44 MAPKs in LPS- and TNF-α-induced CD44-HA binding, we investigated both the phosphorylation of p38 or p42/44 ERKs and the effects of their inhibitors on LPS- and TNF-α-induced CD44-HA binding. SB202190 and PD98059 inhibited both the LPS- and TNF-α-induced phosphorylation of p38 and p42/44 MAPKs, respectively (Fig. 5). In contrast to the normal monocytes (Fig. 3), treatment of LPS-stimulated THP-1 cells with SB202190 did not inhibit CD44-HA binding. However, PD98059 significantly inhibited CD44-HA binding at doses as low as 10 μM (Fig. 6A). Because LPS treatment of THP-1 cells results in the production of endogenous TNF-α, we examined whether SB202190 or PD98059 affected TNF-α expression. Supernatants from cells treated with either SB202190 or PD98059 obtained from the above mentioned experiments were analyzed for TNF-α production. The results show that in contrast to SB202190, PD98059 significantly inhibited LPS-induced TNF-α production at doses as low as 1 μM (Table II).

The above results suggested that the induction of CD44-mediated HA binding observed in LPS-stimulated THP-1 cells is mediated by endogenously produced TNF-α via the activation of p42/44 MAPK. This was confirmed by analysis of CD44-HA binding in LPS-stimulated cells in which TNF-α production was blocked by graded doses of PD98059 followed by reconstitution with a constant concentration of TNF-α (10 ng/ml) (Fig. 6B). Alternatively, LPS-stimulated cells in which TNF-α production was blocked by pretreatment with a constant dose of 25 μM PD98059, could bind HA when exposed to increasing concentrations of TNF-α (0.01–10 ng/ml) (Fig. 6B). The results show that the exogenous addition of TNF-α reversed the PD98059-induced inhibition of CD44-HA binding, suggesting that LPS-activated p42/44 MAPK may play a critical role in TNF-α production, which in turn may induce CD44-mediated HA binding.

Studies performed to delineate the involvement of MAPKs in TNF-α-induced CD44-HA binding revealed that, similar to the results observed in normal monocytes, CD44-mediated HA binding in THP-1 cells was regulated by p38 MAPK. Treatment of THP-1 cells with SB202190 prior to stimulation with TNF-α resulted in inhibition of CD44-mediated HA binding in a dose-dependent manner (Fig. 6C). In contrast, TNF-α-induced CD44-HA binding was not affected by either SB202474 (data not shown) or PD98059 (Fig. 6C). These results suggest that although LPS-induced CD44-HA binding is dependent on endogenously produced TNF-α via p42/44 activation, TNF-α-induced CD44-mediated HA binding requires the activation of p38 MAPK.

**Involvement of Sialidase in the Regulation of LPS- and TNF-α-induced CD44-HA Binding**—It was recently shown that an
inducible lysosomal sialidase could regulate LPS-induced CD44-HA binding in THP-1 cells (22). In view of our results suggesting the involvement of p38 and p42/44 MAPK in TNF-α- and LPS-induced CD44-HA-binding, respectively, we hypothesized that LPS- and TNF-α-induced CD44-HA binding is regulated by sialidase, whose activity may be influenced by the MAPK signal transduction pathway. Therefore, we first investigated whether LPS- and TNF-α-induced CD44-HA-binding requires the induction of sialidase activity. We treated normal monocytes and THP-1 cells with increasing doses of purified sialidase in order to determine whether desialidation of the preexisting surface CD44 could induce an increase in CD44-HA binding. The results show that doses as low as 0.1 units/ml of sialidase were sufficient to induce CD44-HA binding in both normal monocytes (Fig. 7A) and THP-1 cells (Fig. 7B). To determine the validity of the sialidase preparation, the sialidase enzyme (0.8 units/ml) was boiled for 10 min prior to incubation with the 51Cr-labeled monocytes as well as THP-1 cells. 

**Fig. 6.** A, effect of p38 and p42/44 MAPK inhibitors on LPS-induced CD44-mediated HA-binding in THP-1 cells. Cells (0.5 × 10^6/ml) were treated with various concentrations of either SB202190 or PD98059 for 2 h prior to stimulation with LPS. After 24 h, cells were analyzed by the HA adhesion assay. Percentages of adherent cells are presented as the mean of triplicate samples ± S.D. The results shown are representative of three experiments. B, effect of exogenous TNF-α on PD98059-and LPS-treated THP-1 cells. Left panel, THP-1 cells (0.5 × 10^6/ml) were pretreated with PD98059 at varying concentrations ranging from 0 to 50 μM for 2 h prior to LPS (1 μg/ml) or LPS and TNF-α (10 ng/ml) stimulation for 24 h. Right panel, THP-1 cells (0.5 × 10^6/ml) were pretreated with 25 μM PD98059 for 2 h prior to LPS (1 μg/ml) and varying doses of TNF-α (0.01–10 ng/ml) stimulation for 24 h. CD44-HA binding was then analyzed by the HA adhesion assay. Percentages of adherent cells are presented as the mean of triplicate samples ± S.D. The results shown are representative of three experiments. C, effect of p38 and p42/44 MAPK inhibitors on TNF-α-induced CD44-mediated HA binding in THP-1 cells. Cells (0.5 × 10^6/ml) were pretreated with either SB202190 or PD98059 at varying concentrations ranging from 0 to 50 μM for 2 h prior to TNF-α (10 ng/ml) stimulation for 24 h. CD44-HA binding was analyzed by the HA adhesion assay. Percentages of adherent cells are presented as the mean of triplicate samples ± S.D. The results shown are representative of three experiments.
cells. The results show that the boiling of sialidase significantly inhibited HA binding as compared with the binding observed with the native sialidase (Fig. 7). We also treated both monocytes and THP-1 cells with 0.8 units/ml of sialidase in the presence and absence of either anti-CD44 antibodies or isotype-matched control antibodies. Anti-CD44 antibodies inhibited HA binding induced by the treatment of cells with sialidase, suggesting that sialidase-induced HA binding is mediated via the CD44-HA interactions (Fig. 7, A and B). To determine whether sialidase inhibitor could neutralize the biological ac-

### Table II

| Treatment                          | TNF-α production following LPS stimulation in the presence of MAPK inhibitor (pg/ml ± SD; n = 5) |
|-----------------------------------|---------------------------------------------------------------------------------------------|
| LPS + SB202190                    | 1772.2 ± 26.4 1428.6 ± 138.5 1707.4 ± 282.1 1837.6 ± 230.7 2156.2 ± 225.6 2006.8 ± 195.1 2677.1 ± 36.4 |
| LPS + PD98059                     | 2293.6 ± 207.1 964.4 ± 29.6 1096.6 ± 24.4 72.1 ± 10.8 73.9 ± 8.4 69.8 ± 69.9 66.1 ± 5.9 |

* p ≤ 0.05. Unstimulated cells produced less than 16 pg/ml of TNF-α in the culture supernatants.

**Fig. 7.** The addition of exogenous sialidase enzyme induces CD44-mediated HA binding in purified normal monocytes (A) and THP-1 cells (B). Left panel, unstimulated monocytes and THP-1 cells (0.5 × 10⁶/ml) were labeled with 51Cr as described under “Experimental Procedures,” following which the cells were treated with increasing doses of sialidase enzyme (0.1–0.8 units/ml) for 30 min. The cells were washed prior to addition to the HA-coated plate. In addition, sialidase-treated cells were incubated in the presence of either anti-CD44 antibodies or isotype-matched control antibodies. Right panel, to determine the validity of the sialidase preparation, the sialidase enzyme (0.8 units/ml) was boiled for 10 min prior to incubation with the 51Cr-labeled cells. To confirm that the sialidase inhibitor could neutralize the biological activity of sialidase preparation, 0.8 units/ml sialidase was treated with either 0.25, 1.0, or 4.0 mM sialidase inhibitor 2-Neu-Ac for 30 min. Percentages of adherent cells are presented as the mean of triplicate samples ± S.D. The results shown are representative of three experiments.
activity of the sialidase preparation, 0.8 units/ml sialidase was treated with either 0.25, 1.0, or 4.0 mM sialidase inhibitor 2-Neu-Ac for 30 min followed by incubation with normal monocytes and THP-1 cells. The results show that concentrations as low as 0.25 mM of 2-Neu-Ac significantly neutralized HA binding in both THP-1 cells (Fig. 7B) and monocytes (Fig. 7A) compared with the binding observed following treatment of cells with the sialidase enzyme alone.

To demonstrate that LPS- and TNF-α-induced sialidase activity enhances CD44-HA binding, normal monocytes and THP-1 cells were treated with sialidase inhibitor, 2-Neu-Ac, for 2 h prior to stimulation with either LPS or TNF-α, followed by analysis of HA binding. The sialidase inhibitor prevented both LPS- and TNF-α-induced CD44-HA binding in a dose-dependent manner in both normal monocytes (Fig. 8A) and THP-1 cells (Fig. 8B).

To further demonstrate that a sialidase activity was induced following stimulation with LPS and TNF-α, we performed a fluorescence-based sialidase activity assay for lysosomal sialidase. Because high numbers of cells are required for determining sialidase activity (15 × 10⁶ cells/time point), we could not analyze sialidase activity in normal monocytes and therefore used THP-1 cells instead. The measurement of lysosomal sialidase activity was optimized using various concentrations of total protein. The maximal sialidase activity was detected when 200 μg of total cellular proteins was used (data not shown). THP-1 cells were stimulated with either LPS or TNF-α for various times followed by measurement of sialidase activity. Induction of sialidase activity was observed as early as 8 h post-treatment, but a statistically significant increase was observed at 12 and 16 h poststimulation (Fig. 9A). To determine whether induction of sialidase activity is associated with HA binding, THP-1 cells were treated with LPS or TNF-α for various times. The induction of sialidase activity was concurrent with the time-dependent activation of CD44-HA binding in response to LPS or TNF-α (Fig. 9B).

Lysosomal sialidase can reside in either the cytosol or bound to plasma or lysosomal membranes (38). To determine whether the LPS- or TNF-α-induced lysosomal sialidase was membrane-bound or in the soluble cytosol fraction, the cells were stimulated with LPS or TNF-α for 16 h. The cell pellet was treated with 1% digitonin. The resulting supernatant constituted the soluble cytosol fraction, whereas the pellet represented the membrane fractions. The membrane fraction was solubilized in a nonionic detergent, 1% Nonidet P-40. The lysosomal sialidase activity was determined in both the cytosol and membrane fractions. The results show that the LPS and TNF-α stimulation of THP-1 cells induced a significant increase in the lysosomal sialidase activity that was localized in the digitonin-solubilized cytosol fraction (Fig. 9C). In contrast, the lysosomal sialidase activity detected in the Nonidet P-40-solubilized membrane fraction was not increased following TNF-α stimulation of THP-1 cells, whereas this activity was reduced in THP-1 cells stimulated with LPS. These results suggest that LPS- and TNF-α-induced lysosomal sialidase activity was localized in the cytosol fraction.

We also determined whether membrane sialidase was induced in response to LPS or TNF-α stimulation of THP-1 cells by using the membrane sialidase-specific substrate, bovine mixed gangliosides type II, as described under “Experimental
The results show that stimulation of THP-1 cells with LPS (Fig. 9D) or TNF-α (data not shown) did not induce the membrane sialidase activity.

**Regulation of CD44-HA Interactions in Human Monocytes**

**TNF-α-induced Sialidase Activity Involves p38 MAPK Activation in THP-1 Cells**—Because induction of sialidase activity was found to be associated with CD44-mediated HA binding, we determined whether LPS- and TNF-α-induced sialidase activity involved the activation of p38 or p42/44 MAPKs. Cells were pretreated with various concentrations of either SB203580 or PD98059 for 2 h prior to stimulation with LPS or TNF-α for 16 h followed by measurement of sialidase activity. In LPS-stimulated cells, PD98059 significantly reduced LPS-induced sialidase activity in a dose-dependent manner, whereas SB203580 did not have any effect (Fig. 10A). However, in TNF-α-stimulated cells, SB203580 significantly reduced TNF-α-induced sialidase activity in a dose-dependent manner, whereas SB203580 and SB202474, the inactive analogue of SB203580, failed to inhibit sialidase activity (Fig. 10B). Taken together, these results suggest that both LPS and TNF-α induce lysosomal sialidase activity in THP-1 cells. Furthermore, the LPS-induced sialidase activity and consequent CD44-HA binding may be regulated by endogenously produced TNF-α through p42/44 activation. In contrast, TNF-α-induced sialidase activity resulting in CD44-mediated HA-binding may be dependent on p38 MAPK activation.

**DISCUSSION**

In this study, we examined the role of MAPK signaling pathways mediating the synthesis of the functionally active, HA-binding form of CD44 in LPS-stimulated human monocytic cells. Our results show that endogenously produced TNF-α mediates LPS-induced CD44-HA binding in human monocytic cells. Furthermore, TNF-α-activated p38 MAPK plays a critical role in the generation of LPS-induced HA-adhesive CD44. We also demonstrated that acquisition of the HA-binding form of CD44 involves sialidase activation in both LPS- and TNF-α-stimulated monocytic cells. Furthermore, p38 MAPK activation was required for TNF-α-induced sialidase activity and consequent CD44-HA binding. Taken together, the results suggest a key role for TNF-α in regulating the generation of HA-adhesive CD44 by inducing sialidase activity through p38 MAPK activation in LPS-stimulated monocytic cells.

Endotoxins and immunoregulatory cytokines have been shown to regulate CD44-mediated HA binding in different cell types. The results of our study suggest that the regulation of CD44-HA interactions by TNF-α and LPS involves the activation of MAPKs, specifically p38 MAPK, which is consistent with previous findings. The activation of MAPKs by LPS and TNF-α leads to the induction of sialidase activity, which in turn facilitates the binding of HA to CD44.

**Fig. 9.** A. LPS and TNF-α induce lysosomal sialidase activity in THP-1 cells. THP-1 cells were treated with either LPS or TNF-α for times ranging from 0 to 20 h. At each time point, cells were pelleted, washed once in phosphate-buffered saline, and analyzed for lysosomal sialidase activity. Results are presented as the mean fluorescence intensity of three separate experiments ± S.D. The increase in activity at 12 and 16 h was determined to be statistically significant (p < 0.01) and is indicated by an asterisk. B. LPS and TNF-α induce a time-dependent induction of HA binding in THP-1 cells. THP-1 cells were treated with either LPS (1 μg/ml) or TNF-α (10 ng/ml) for times ranging from 0 to 48 h. Cells were analyzed for HA binding by the HA adhesion assay. Percentages of adherent cells are presented as the mean of triplicate samples ± S.D. The increase in activity at 12 and 16 h was determined to be statistically significant (p < 0.01) and is indicated by an asterisk. C. LPS- and TNF-α-induced lysosomal sialidase activity is localized in the cytosol. THP-1 cells (15 × 10⁶ cells/ml) were stimulated with LPS (1 μg/ml) or TNF-α (10 ng/ml) for 16 h. The cell pellet was treated with 1% digitonin. Following centrifugation, the supernatant constituted the soluble cytosol fraction, whereas the pellet represented the membrane fractions. The membrane fraction was solubilized in a nonionic detergent, 1% Nonidet P-40. The lysosomal sialidase activity was determined in the cytosol and membrane fractions by fluorometry. Results are presented as the mean fluorescence intensity of three separate experiments ± S.D. The increase in activity at 12 and 16 h was determined to be statistically significant (p < 0.01) and is indicated by an asterisk. D. LPS and TNF-α induce lysosomal sialidase activity. In LPS-stimulated cells, PD98059 significantly reduced LPS-induced sialidase activity, whereas SB202190 did not have any effect (Fig. 10). In contrast, TNF-α-stimulated cells, SB202190 significantly reduced TNF-α-induced sialidase activity in a dose-dependent manner, whereas SB202474, the inactive analogue of SB202190, failed to inhibit sialidase activity (Fig. 10B). Taken together, these results suggest that both LPS and TNF-α induce lysosomal sialidase activity in THP-1 cells. Furthermore, the LPS-induced sialidase activity and consequent CD44-HA binding may be regulated by endogenously produced TNF-α through p42/44 activation. In contrast, TNF-α-induced sialidase activity resulting in CD44-mediated HA-binding may be dependent on p38 MAPK activation.

**DISCUSSION**

In this study, we examined the role of MAPK signaling pathways mediating the synthesis of the functionally active, HA-binding form of CD44 in LPS-stimulated human monocytic cells. Our results show that endogenously produced TNF-α mediates LPS-induced CD44-HA binding in human monocytic cells. Furthermore, TNF-α-activated p38 MAPK plays a critical role in the generation of LPS-induced HA-adhesive CD44. We also demonstrated that acquisition of the HA-binding form of CD44 involves sialidase activation in both LPS- and TNF-α-stimulated monocytic cells. Furthermore, p38 MAPK activation was required for TNF-α-induced sialidase activity and consequent CD44-HA binding. Taken together, the results suggest a key role for TNF-α in regulating the generation of HA-adhesive CD44 by inducing sialidase activity through p38 MAPK activation in LPS-stimulated monocytic cells.

Endotoxins and immunoregulatory cytokines have been shown to regulate CD44-mediated HA binding in different cell types.
The regulation of CD44-HA interactions in human monocytes involves the signal transduction pathways involved in the generation of the HA-binding form of CD44. LPS is responsible for the induction of several pro- and anti-inflammatory cytokines in monocytic cells (44). LPS exerts its effects following its association with the plasma-LPS-binding protein and the binding of this complex with the CD14-Toll-like receptor-4 complex (44, 45, 48, 49). Therefore, regulation of LPS-induced CD44-mediated HA binding in monocytic cells may involve a combination of signals delivered by the interaction of LPS with the CD14-Toll-like receptor complex as well as by the interaction of endogenously produced cytokines with their cognate receptors. Our results suggest that LPS-induced CD44-mediated HA binding in primary monocytes may be regulated by the endogenously produced TNF-α and IL-10.

To understand the molecular mechanism underlying the regulation of CD44-HA binding in LPS-stimulated monocytic cells, we examined the role of p38 and ERK MAPKs. Our results consistently demonstrated a significant inhibition of LPS- and TNF-α-induced HA binding by the p38 inhibitor, SB202190, in normal monocytes. The inhibition of HA binding by SB202190 was specific, since these effects were observed at concentrations ranging from 5 to 10 μM, thus virtually excluding their nonspecific effects (50). However, IL-10 induction of the HA-binding form of CD44 did not involve either p38 or p42/44 MAPK activation. IL-10 has been shown to mediate its inhibitory effects by inducing the expression of the cell cycle inhibitor cyclin-dependent kinase inhibitor p19INK4D in macrophages (16, 17, 24, 28, 39, 47). However, very little is known about the signal transduction pathways involved in the generation of the HA-binding form of CD44. LPS is responsible for the induction of several pro- and anti-inflammatory cytokines in monocytic cells (44). LPS exerts its effects following its association with the plasma-LPS-binding protein and the binding of this complex with the CD14-Toll-like receptor-4 complex (44, 45, 48, 49). Therefore, regulation of LPS-induced CD44-mediated HA binding in monocytic cells may involve a combination of signals delivered by the interaction of LPS with the CD14-Toll-like receptor complex as well as by the interaction of endogenously produced cytokines with their cognate receptors. Our results suggest that LPS-induced CD44-mediated HA binding in primary monocytes may be regulated by the endogenously produced TNF-α and IL-10.

To understand the molecular mechanism underlying the regulation of CD44-HA binding in LPS-stimulated monocytic cells, we examined the role of p38 and ERK MAPKs. Our results consistently demonstrated a significant inhibition of LPS- and TNF-α-induced HA binding by the p38 inhibitor, SB202190, in normal monocytes. The inhibition of HA binding by SB202190 was specific, since these effects were observed at concentrations ranging from 5 to 10 μM, thus virtually excluding their nonspecific effects (50). However, IL-10 induction of the HA-binding form of CD44 did not involve either p38 or p42/44 MAPK activation. IL-10 has been shown to mediate its inhibitory effects by inducing the expression of the cell cycle inhibitor cyclin-dependent kinase inhibitor p19INK4D in macrophages (16, 17, 24, 28, 39, 47). However, very little is known about the signal transduction pathways involved in the generation of the HA-binding form of CD44. LPS is responsible for the induction of several pro- and anti-inflammatory cytokines in monocytic cells (44). LPS exerts its effects following its association with the plasma-LPS-binding protein and the binding of this complex with the CD14-Toll-like receptor-4 complex (44, 45, 48, 49). Therefore, regulation of LPS-induced CD44-mediated HA binding in monocytic cells may involve a combination of signals delivered by the interaction of LPS with the CD14-Toll-like receptor complex as well as by the interaction of endogenously produced cytokines with their cognate receptors. Our results suggest that LPS-induced CD44-mediated HA binding in primary monocytes may be regulated by the endogenously produced TNF-α and IL-10.

To understand the molecular mechanism underlying the regulation of CD44-HA binding in LPS-stimulated monocytic cells, we examined the role of p38 and ERK MAPKs. Our results consistently demonstrated a significant inhibition of LPS- and TNF-α-induced HA binding by the p38 inhibitor, SB202190, in normal monocytes. The inhibition of HA binding by SB202190 was specific, since these effects were observed at concentrations ranging from 5 to 10 μM, thus virtually excluding their nonspecific effects (50). However, IL-10 induction of the HA-binding form of CD44 did not involve either p38 or p42/44 MAPK activation. IL-10 has been shown to mediate its inhibitory effects by inducing the expression of the cell cycle inhibitor cyclin-dependent kinase inhibitor p19INK4D in macrophages (16, 17, 24, 28, 39, 47). However, very little is known about the signal transduction pathways involved in the generation of the HA-binding form of CD44. LPS is responsible for the induction of several pro- and anti-inflammatory cytokines in monocytic cells (44). LPS exerts its effects following its association with the plasma-LPS-binding protein and the binding of this complex with the CD14-Toll-like receptor-4 complex (44, 45, 48, 49). Therefore, regulation of LPS-induced CD44-mediated HA binding in monocytic cells may involve a combination of signals delivered by the interaction of LPS with the CD14-Toll-like receptor complex as well as by the interaction of endogenously produced cytokines with their cognate receptors. Our results suggest that LPS-induced CD44-mediated HA binding in primary monocytes may be regulated by the endogenously produced TNF-α and IL-10.

To understand the molecular mechanism underlying the regulation of CD44-HA binding in LPS-stimulated monocytic cells, we examined the role of p38 and ERK MAPKs. Our results consistently demonstrated a significant inhibition of LPS- and TNF-α-induced HA binding by the p38 inhibitor, SB202190, in normal monocytes. The inhibition of HA binding by SB202190 was specific, since these effects were observed at concentrations ranging from 5 to 10 μM, thus virtually excluding their nonspecific effects (50). However, IL-10 induction of the HA-binding form of CD44 did not involve either p38 or p42/44 MAPK activation. IL-10 has been shown to mediate its inhibitory effects by inducing the expression of the cell cycle inhibitor cyclin-dependent kinase inhibitor p19INK4D in macrophages (16, 17, 24, 28, 39, 47). However, very little is known about the signal transduction pathways involved in the generation of the HA-binding form of CD44. LPS is responsible for the induction of several pro- and anti-inflammatory cytokines in monocytic cells (44). LPS exerts its effects following its association with the plasma-LPS-binding protein and the binding of this complex with the CD14-Toll-like receptor-4 complex (44, 45, 48, 49). Therefore, regulation of LPS-induced CD44-mediated HA binding in monocytic cells may involve a combination of signals delivered by the interaction of LPS with the CD14-Toll-like receptor complex as well as by the interaction of endogenously produced cytokines with their cognate receptors. Our results suggest that LPS-induced CD44-mediated HA binding in primary monocytes may be regulated by the endogenously produced TNF-α and IL-10.

To understand the molecular mechanism underlying the regulation of CD44-HA binding in LPS-stimulated monocytic cells, we examined the role of p38 and ERK MAPKs. Our results consistently demonstrated a significant inhibition of LPS- and TNF-α-induced HA binding by the p38 inhibitor, SB202190, in normal monocytes. The inhibition of HA binding by SB202190 was specific, since these effects were observed at concentrations ranging from 5 to 10 μM, thus virtually excluding their nonspecific effects (50). However, IL-10 induction of the HA-binding form of CD44 did not involve either p38 or p42/44 MAPK activation. IL-10 has been shown to mediate its inhibitory effects by inducing the expression of the cell cycle inhibitor cyclin-dependent kinase inhibitor p19INK4D in macrophages (16, 17, 24, 28, 39, 47). However, very little is known about the signal transduction pathways involved in the generation of the HA-binding form of CD44. LPS is responsible for the induction of several pro- and anti-inflammatory cytokines in monocytic cells (44). LPS exerts its effects following its association with the plasma-LPS-binding protein and the binding of this complex with the CD14-Toll-like receptor-4 complex (44, 45, 48, 49). Therefore, regulation of LPS-induced CD44-mediated HA binding in monocytic cells may involve a combination of signals delivered by the interaction of LPS with the CD14-Toll-like receptor complex as well as by the interaction of endogenously produced cytokines with their cognate receptors. Our results suggest that LPS-induced CD44-mediated HA binding in primary monocytes may be regulated by the endogenously produced TNF-α and IL-10.

To understand the molecular mechanism underlying the regulation of CD44-HA binding in LPS-stimulated monocytic cells, we examined the role of p38 and ERK MAPKs. Our results consistently demonstrated a significant inhibition of LPS- and TNF-α-induced HA binding by the p38 inhibitor, SB202190, in normal monocytes. The inhibition of HA binding by SB202190 was specific, since these effects were observed at concentrations ranging from 5 to 10 μM, thus virtually excluding their nonspecific effects (50). However, IL-10 induction of the HA-binding form of CD44 did not involve either p38 or p42/44 MAPK activation. IL-10 has been shown to mediate its inhibitory effects by inducing the expression of the cell cycle inhibitor cyclin-dependent kinase inhibitor p19INK4D in macrophages (16, 17, 24, 28, 39, 47). However, very little is known about the signal transduction pathways involved in the generation of the HA-binding form of CD44. LPS is responsible for the induction of several pro- and anti-inflammatory cytokines in monocytic cells (44). LPS exerts its effects following its association with the plasma-LPS-binding protein and the binding of this complex with the CD14-Toll-like receptor-4 complex (44, 45, 48, 49). Therefore, regulation of LPS-induced CD44-mediated HA binding in monocytic cells may involve a combination of signals delivered by the interaction of LPS with the CD14-Toll-like receptor complex as well as by the interaction of endogenously produced cytokines with their cognate receptors. Our results suggest that LPS-induced CD44-mediated HA binding in primary monocytes may be regulated by the endogenously produced TNF-α and IL-10.
phages through STAT3 activation (51–53). Determination of whether IL-10-induced CD44-HA binding is regulated by STAT3 activation requires investigation.

The role of MAPKs in LPS- and TNF-α-induced CD44-HA interactions could not be precisely studied in normal monocytes, because endogenously produced IL-10 up-regulated HA binding independently of p38 and ERKs. IL-10-refractory THP-1 cells provided a useful model to study the LPS-induced signaling events in CD44-mediated HA-binding without the additional effects of endogenous IL-10. In THP-1 cells, endogenously produced TNF-α was found to play a critical role in LPS-induced CD44-mediated HA binding. Contrary to the results obtained with normal monocytes, LPS-induced TNF-α production and CD44-mediated HA binding in THP-1 cells were regulated by p42/44 MAPK. Since LPS-induced TNF-α production in these cells was inhibited by PD98059, a p42/44 inhibitor, the effects of this inhibitor on LPS-induced HA binding were attributed to the blockade of endogenously produced TNF-α. This was confirmed by the exogenous addition of TNF-α to the THP-1 cells in which LPS-induced TNF-α production was simultaneously blocked by PD98059. The fact that exogenous TNF-α restored CD44-HA binding suggested that LPS-induced CD44-HA binding observed in THP-1 cells is regulated by endogenously produced TNF-α via the activation of p42/44 MAPK. However, TNF-α-induced CD44-HA binding in THP-1 cells was found to be dependent on p38 activation.

Activation of p38 MAPK has been shown to play a role in the regulation of cellular and cytokine genes (33, 34, 54). For example, T cell activation with specific antigen or staphylococcal antigens has been shown to induce p38 activation, resulting in TNF-α production (43). It has also been demonstrated that p38 MAPK regulates IL-1 (43), IL-6 (55), TNF-α (43), and IL-10 (35) production in human monocytes through the activation of the CD14 receptor. We have observed differential involvement of p38 and p42/44 MAPKs in LPS-induced TNF-α production in normal monocytes and THP-1 cells, respectively. The molecular mechanisms for the observed differences in TNF-α regulation in THP-1 cells and primary monocytes are not clear at present but may be attributed to the tumorigenic nature of THP-1 cells. However, both p38 and p42/44 MAPKs have been shown to regulate TNF-α expression, depending on the cell types and stage of cell differentiation/maturity (56, 57).

The CD44 molecule possesses five N- and O-linked glycosylation sites in its extracellular domain (58) so that the biological functions of CD44 may be regulated by post-translational modifications such as glycosylation (13, 19–21). Sialidation of CD44 has been shown to regulate CD44-HA binding of LPS-stimulated monocytic cells (22). Sialidase is a key enzyme in the metabolism of glycoproteins and glycolipids. Mammalian sialidases have been classified into three types depending on subcellular localization and enzymatic properties. These types, located in the cytosol, in lysosomes, and in plasma membranes, have been cloned and characterized (40, 59, 60). Lysosomal sialidases catalyze the intralysosomal degradation of sialoglycoconjugates by releasing terminal sialic acids from their oligosaccharide chains (61). The desialylation of these glycoconjugates is a crucial event leading to the modulation of cellular functions in numerous physiological and pathological processes. Aberrant sialylation in cancer cells is generally accepted as a characteristic feature associated with metastatic potential (62). Human lysosomal sialidase has also been shown to be involved in two genetically distinct lysosomal storage disorders, sialidosis and galactosialidosis (60, 63).

In this study, we show that LPS-induced as well as TNF-α-induced CD44-HA binding may involve the activation of lysosomal sialidase in both normal monocytes and THP-1 cells. We have also shown that LPS- and TNF-α-induced lysosomal sialidase activity resides in the cytosol fraction and not in the cell membranes. Furthermore, the ganglioside-specific membrane sialidases are not induced in THP-1 cells following stimulation with either LPS or TNF-α. There is little information regarding the involvement of signaling molecules, particularly MAPKs, in regulating sialidase activity. Calcium activation has been shown to stimulate sialidase activity; for example, chelators (EGTA) and certain metals (Cu²⁺/Zn²⁺) have been shown to inhibit some sialidases (64). Herein, we show for the first time that TNF-α-induced sialidase activity is regulated by p38 MAPK in THP-1 cells. In contrast, LPS-induced sialidase activity was dependent on p42/44 MAPK-mediated TNF-α production. Blocking TNF-α production by PD98059, a p42/44 inhibitor, significantly reduced the LPS-induced sialidase activity and CD44-HA binding. Subsequently, TNF-α-mediated p38 MAPK activation induced sialidase activity and CD44-HA binding. These results suggest that although LPS-induced CD44-HA binding is dependent on endogenously produced TNF-α via p42/44 activation, TNF-α-induced sialidase activity resulting in CD44-mediated HA binding may require p38 MAPK activation. Although our data suggest a prominent role for sialidase activity in the generation of HA-adhesive CD44, the contribution of other factors such as sulfation of CD44 (65, 66) cannot be ruled out in our model system and needs to be investigated.

We have previously demonstrated that LPS- and TNF-α-induced CD44 expression is not mediated by p38 MAPK activation (28). Since the results of this study show the involvement of p38 MAPK in LPS- and TNF-α-induced CD44-mediated HA binding, these observations suggest that the induction of CD44 expression and the generation of a CD44 form capable of binding HA are two independent events that are regulated by distinct MAPK signaling pathways. Induction of CD44 expression may involve the activation of JNK MAPK (28), whereas activation of p38 MAPK may be required for the generation of HA-adhesive CD44 in LPS-stimulated monocytic cells. This conclusion is based on the observations that low concentrations of SB202190 inhibited LPS- and TNF-α-induced CD44-HA binding (Fig. 3), but not CD44 expression (28), in normal monocytes. Furthermore, PD98059 completely inhibited LPS-induced CD44-HA binding in THP-1 cells (Fig. 6) without any effect on CD44 expression (28) (data not shown). Conversely, SB202190 at high doses partially inhibited LPS-induced CD44 expression (28) without any effect on HA binding (Fig. 6). In summary, our results show for the first time the involvement of p38 MAPK in the generation of HA-adhesive, functionally active CD44 in LPS- and TNF-α-stimulated monocytic cells. Using THP-1 cells as a model system, we provide evidence that TNF-α-induced CD44-HA binding may involve the induction of sialidase activity, which is regulated by p38 MAPK. These studies provide a basis for the identification of molecular players in CD44 induction and CD44-mediated HA binding that may help in designing targeted drug therapy for inflammatory diseases.

Acknowledgments—Drs. John Webb, Marko Kryworuchko, and Gina Graziani-Bowering are gratefully acknowledged for critically reading the manuscript.

REFERENCES
1. Naor, D., Sionov, R. V., and Ish-Shalom, D. (1997) Adv. Cancer Res. 71, 241–319
2. Miyake, K., Underhill, C. B., Lesley, J., and Kincade, P. W. (1990) J. Exp. Med. 172, 69–75
3. Siegelman, M. H., DeGrendele, H. C., and Estess, P. (1999) J. Leukocyte Biol. 66, 315–321
4. Wittig, B., Schwarzer, C., Fehr, N., Gunthert, U., and Zoller, M. (1998) J. Immunol. 161, 1069–1073
5. Brocke, S., Piercy, C., Steinman, L., Weissman, I. L., and Verma, T. (1999)
