Differential induction of CD38 expression by TNF-\(\alpha\) in asthmatic airway smooth muscle cells

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Jude JA, Solway J, Panettieri RA Jr, Walseth TF, Kannan MS. Differential induction of CD38 expression by TNF-\(\alpha\) in asthmatic airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 299: L879–L890, 2010. First published August 6, 2010; doi:10.1152/ajplung.00021.2010.—The ADP-ribosyl cyclase activity of CD38, a membrane protein expressed in human airway smooth muscle cells (ASM) and ASM-derived cells, generates cyclic ADP-ribose (cADPR), a Ca\(^{2+}\)-mobilizing agent. cADPR-mediated Ca\(^{2+}\) responses to agonists are augmented in human ASM cells by TNF-\(\alpha\). CD38-deficient mice fail to develop airway hyperresponsiveness following intranasal TNF-\(\alpha\) or IL-13 challenge, suggesting a role in asthma. The role of CD38 in human asthma remains unknown. We hypothesized that CD38 expression will be elevated in ASM cells from asthmatic donors (ASMA cells). CD38 mRNA and ADP-ribosyl cyclase activity were measured in cells maintained in growth-arrested conditions and exposed to vehicle or TNF-\(\alpha\) (10–40 ng/ml). TNF-\(\alpha\)-induced induction of CD38 expression was greater in ASMA than in ASM cells from nonasthmatic donors (ASMA). In four of the six donors, basal and TNF-\(\alpha\)-induced ERK and p38 MAPK activation were higher in ASMA than ASM cells. NK4 MAPK activation was lower in ASMA than ASMA cells. Nuclear NF-\(\kappa\)B (p50 subunit) and phosphorylated c-Jun were comparable in cells from both groups, although nuclear c-Fos (part of the AP-1 complex) levels were lower in ASMA than ASMA cells. NF-\(\kappa\)B or AP-1 binding to their consensus sequences was comparable in ASMA and ASM cells, as are the decay kinetics of CD38 mRNA. The findings suggest that the differential induction of CD38 by TNF-\(\alpha\) in ASMA cells is due to increased transcriptional regulation involving ERK and p38 MAPK activation and is independent of changes in NF-\(\kappa\)B or AP-1 activation. The findings suggest a potential role for CD38 in the pathophysiology of asthma.

ADP-ribosyl cyclase; MAP kinases; nuclear factor-\(\kappa\)B; smooth muscle

ASTHMA IS AN AIRWAY INFLAMMATORY disorder with a complex etiology, characterized by airway hyperresponsiveness (AHR), inflammation, and hypersecretion of mucus into the airway lumen, resulting in reversible difficulty in breathing. Airway smooth muscle (ASM) plays a central role in the pathogenesis of asthma (27). Several reports have provided evidence for phenotypic changes in ASM cells from asthmatic individuals. These changes are characterized by increased rate of cell proliferation, increased ability to develop mechanical force, increased secretion of chemokines, and changes in signal transduction pathways that contribute to some of the phenotypic changes (5, 23, 29, 30, 34). The increase in ASM mass and the hypercontractile nature of the ASM in airways of asthmatic individuals largely contribute to the airway narrowing during asthma attacks. In addition to the mechanical role, ASM also plays an important role in airway inflammation (7, 31, 34). Growth factors (VEGF, TGF-\(\beta\), and PDGF), cytokines (granulocyte-macrophage colony-stimulating factor and IL-13), and other chemokines (eotaxins, IL-6, and IL-8) secreted by ASM cells act in an autocrine fashion to elicit pathological changes associated with asthma (21).

CD38 is a 45-kDa transmembrane protein expressed in airway myocytes (39). This protein possesses multifunctional enzyme activities to metabolize NAD, a by-product of cellular energy metabolism. The ADP-ribosyl cyclase activity of CD38 converts NAD to cyclic ADP-ribose (cADPR), whereas the cADPR hydrolase activity of the protein converts the cADPR to ADP-ribose (ADPR) (20). Among these products, cADPR is known to release Ca\(^{2+}\) from the sarcoplasmic reticulum in smooth muscle cells (33). Therefore, the expression of CD38 and the regulation of its cyclase and hydrolase activities are considered important for intracellular Ca\(^{2+}\) regulation in airway myocytes. We previously demonstrated that downregulation of CD38 expression using antisense oligonucleotides attenuates agonist-induced Ca\(^{2+}\) responses in cultured human airway myocytes (24). Furthermore, airway myocytes from CD38\(^{-/-}\) mice exhibit attenuated intracellular Ca\(^{2+}\) responses to agonists compared with myocytes from wild-type mice (10). In vivo studies using murine models of AHR showed that CD38\(^{-/-}\) mice develop a significantly lower magnitude of AHR than CD38\(^{+/+}\) mice (14–16). While evidence supports a role for CD38 in the development of AHR in mouse models, the potential role of CD38 in the pathophysiology of human inflammatory airway disorders, such as asthma, remains unknown. Since CD38 has a pivotal role in intracellular Ca\(^{2+}\) dynamics and contractility of ASM, CD38 expression and function may be modulated in asthmatic airways. In human ASM (HASM) cells, we previously reported that TNF-\(\alpha\) increases CD38 expression and cyclase activity and that the effects of TNF-\(\alpha\) are mediated through activation of the MAPKs and the transcription factors NF-\(\kappa\)B and AP-1 (9, 25, 37). Furthermore, increased TNF-\(\alpha\) levels have been reported within the lungs of asthmatic patients (4, 40). In animal models of AHR, previous studies showed that inhibitors of MAPKs, as well as NF-\(\kappa\)B and AP-1, attenuate AHR following allergen sensitization and challenge (11, 12, 19).
Findings of a recent study suggest that inhibition of p38 MAPK may increase glucocorticoid sensitivity in patients with severe asthma (3). In the present study, we hypothesize that increased TNF-α signaling will augment CD38 expression in HASM cells isolated from patients with asthma. To test this hypothesis, CD38 expression at mRNA and protein levels, MAPK activation, and nuclear localization and activation of the transcription factors NF-κB and AP-1 were determined in HASM cells from nonasthmatic and asthmatic donors under basal and TNF-α-stimulated conditions. In light of reports in other cell systems of an interaction between NF-κB and CCAAT/enhancer-binding protein (C/EBPβ) (6, 22, 32, 41), we also investigated C/EBPβ levels in HASM cells from asthmatic and nonasthmatic donors under basal and TNF-α-stimulated conditions.

MATERIALS AND METHODS

Reagents. Tris base, glucose, HEPES, dexamethasone, and other chemicals were purchased from Sigma Chemical (St. Louis, MO) unless otherwise noted; recombinant human TNF-α (rhTNF-α) from R & D Systems (Minneapolis, MN); HBSS and DMEM from Gibco-BRL (Grand Island, NY); TRIzol, Superscript III reverse transcriptase, and 100-bp DNA ladder from Invitrogen (Carlsbad, CA); chemiluminescent substrate for horseradish peroxidase (HRP) from Millipore (Billerica, MA); SYBR Green Master Mix from Stratagene (La Jolla, CA), and the default threshold of the machine was used to determine the cycle threshold (Ct) value. The fold change of CD38 mRNA expression was determined using the ΔΔCt method.

ADP-ribosyl cyclase and cADPR hydrolase assays. Whole cell lysates were collected from ASMA and ASMNA cells after 24 h of exposure (vehicle or rhTNF-α (10–40 ng/ml)). The ADP-ribosyl cyclase activity of HASM cell lysates was quantified by measurement of the reverse cyclase activity of CD38 (37). HASM whole cell lysates containing 5 μg of total protein were incubated for 1 h at 37°C with or without 10 mM nicotinamide in the presence of 0.45 mM cADPR. The reverse cyclase reaction was terminated by addition of 0.25 μl of 1 M HCl, and the cells were vacuum-filtered through a protein-binding membrane (0.45 μm; Immobilon, Millipore) and neutralized with 15 μl of 2 M Tris-base. The filtrate was incubated with reagent mixture containing 2 μM resazurin, 0.76% (vol/vol) ethanol, 4 μM flavin mononucleotide, 40 μg/ml alcohol dehydrogenase, and 0.04 U/ml diaphorase in NaH2PO4/Na2HPO4 buffer, pH 6.8, at room temperature. The fluorescence was quantified in a fluorometer (FLUO Star Galaxy, BMG Bitechnologies, Cary, NC), and the rate of fluorescence emission at 590 nm following excitation at 544 nm was calculated. Known NAD standards were used in the resazurin assay to quantify the NAD generated in the reverse cyclase reaction.

cADPR hydrolase activity was determined by incubation of cell lysates (10 μg) with 200 μM cADPR and 32P-labeled cADPR (11,000 cpm/reaction) for 90 min at room temperature in a 30-μl reaction volume. One microliter of the reaction was spotted on a cellulose-coated TLC plate to separate the reaction products in a solution containing ethanol and 2 M sodium formate. The TLC plates were air-dried and exposed to PhosphorImager screens overnight and developed in Cyclone PhosphorImager developer (PerkinElmer, Waltham, MA) to visualize the resolved products. Densitometry analysis was performed on the images using Optiquant image analysis software (PerkinElmer) to determine the proportion of 32P-labeled ADPγS generated from the total 32P-labeled cADPR substrate. The initial input of cADPR in the hydrolysis reaction (2,000 pmol) was used to calculate the picomoles of ADPγS generated by each sample.

Western blot detection of MAPKs and transcription factors. Activation of ERK, p38, or JNK MAPKs was determined by exposure of the cells to TNF-α (10 ng/ml) for 5 min and collection of the whole cell lysates in PBS. The cell pellets were lysed in lysis buffer (20 mM Tris, 250 mM sucrose, 200 mM NaCl, 1 mM NaF, 1 mM NaVO4, and protease inhibitor cocktail) by sonication on ice. NF-κB activation was determined by treatment of cells with TNF-α for 1 h and collection of the nuclear extracts using the NE-PER cell nuclear extraction kit (Thermo Scientific, Rockford, IL). Ten micrograms of total protein were resolved in a 10–20% Tris-HCl SDS gel and electrophoretically transferred onto a polyvinylidene difluoride membrane. The blot was blocked in 5% skim milk solution in PBS containing 0.05% Tween 20 for 4 h. The blot was probed with antibodies against phosphorylated MAPKs (phosphorylated ERK1/2, phosphorylated p38, and phosphorylated JNK), the respective total MAPKs, or p50 (NF-κB subunit), phosphorylated c-Jun, c-Fos, or C/EBPβ and then incubated with HRP-conjugated secondary antibodies for 1 h. The blots were treated with rhTNF-α (10 ng/ml) for 24 h, and total cellular RNA was isolated using TRIzol. An equal quantity (500 ng) of total RNA from each sample was used in a reverse transcription reaction using the Superscript III reverse transcriptase kit. The following primer sets were used in RT-PCR or quantitative RT-PCR: 5'-ACAAACCCCTGCTGCCGCTCT-3' (forward) and 5'-GACAT-CGCGGACAGCGTCT-3' (reverse) for human CD38 (CDUP99), 5'-GAAGGGAAGTGGCAGGT-3' (reverse) for GAPDH, and 5'-CACGGAGAT-GGTTTAGTCTG-3' (forward) and 5'-CACAGACCCTTTGCT-CCACTT-3' (reverse) for TNF-α receptor 1 (TNFR1). Quantitative RT-PCR was performed using Brilliant SYBR Green Master Mix under the following conditions: denaturation at 94°C for 5 min, 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s, and 1 cycle of 94°C for 30 s and 60°C for 30 s to generate the melting curve. Fluorescence was measured in a real-time thermocycler (model Mx3000P, Stratagene, La Jolla, CA), and the default threshold of the machine was used to determine the cycle threshold (Ct) value. The fold change of CD38 mRNA expression was determined using the ΔΔCt method.
washed in PBS containing 0.05% Tween 20 and then treated with the chemiluminescent substrate for HRP and exposed to X-ray film for visualization of the bands.

**Determination of CD38 mRNA turnover.** Decay rate of TNF-α-induced CD38 mRNA was determined by treatment of HASM cells with rhTNF-α (10 ng/ml) for 24 h and return of the cells to fresh medium. Total RNA was collected at 0, 12, and 24 h after removal of TNF-α, and cDNA was synthesized as described above. Quantitative RT-PCR was performed using the cDNA with primers to amplify CD38 and GAPDH sequences. The relative abundance of CD38 mRNA at each time point was determined by the ΔΔCt method. In a variation of the above-described experiment, HASM cells were treated with rhTNF-α (10 ng/ml) for 12 h. The cells were washed to remove TNF-α, and further transcription was arrested by addition of actinomycin D (5 μg/ml). Total RNA was collected 0, 1, 3, and 12 h after the arrest of transcription and converted to cDNA. Relative abundance of CD38 mRNA at each time point was determined by quantitative RT-PCR. 

**EMSA.** Double-stranded NF-κB consensus oligonucleotide was end-labeled with [γ-^32^P]ATP (3,000 Ci/mmol at 10 μCi/ml) using T4 polynucleotide kinase (Promega) according to the manufacturer’s instructions. Five micrograms of nuclear extracts from HASM cells treated with vehicle or TNF-α (10 ng/ml) for 1 h were incubated with 2 pmol of labeled oligonucleotide in a binding buffer [20% glycerol, 5 mM MgCl₂, 2.5 mM EDTA, 2.5 mM DTT, 250 mM NaCl, 50 mM Tris-HCl (pH 7.5), and 0.25 mg/ml poly(dI-dC)]. The binding reaction was resolved in a nonreducing, 4% polyacrylamide gel using 0.5 M Tris-borate-EDTA as a running buffer. For visualization of the DNA-protein complexes in the gels, the air-dried gels were exposed to a PhosphorImager screen, which was developed with a Cyclone PhosphorImager developer.

**ELISA.** ELISA was performed to determine the NF-κB or AP-1 activation according to the manufacturer’s instructions. Briefly, 3 μg of nuclear extracts from HASM cells were incubated in a multiwell plate coated with oligonucleotides carrying consensus NF-κB or AP-1 sequences. Specificity of the binding was determined by addition of 20 pmol (20× excess) of competitor oligonucleotide to some of the reactions.

**Data analysis.** We used cells isolated from nine asthmatic and nine nonasthmatic donors; n refers to the number of samples (i.e., number of donors). The data from enzymatic activities, mRNA fold changes, and densitometry values of Western blots are expressed as means ± SE and were statistically analyzed by Student’s t-test or one-way ANOVA (with Bonferroni’s posttest comparison), when applicable, using GraphPad Prism software. The differences were considered significant when P ≤ 0.05. In mRNA decay rate experiments, the relative abundance of CD38 mRNA is expressed as percentage of CD38 mRNA at time 0.

**RESULTS**

**CD38 expression in HASM cells.** HASM cells maintained in a growth-arrested condition were treated with a range of rhTNF-α concentrations (10–40 ng/ml) for 24 h, and CD38 mRNA expression was determined by RT-PCR and ADP-ribosyl cyclase activity. There was no detectable CD38 mRNA expression or ADP-ribosyl cyclase activity in HASM cells from ASMNA or ASMA cells under basal conditions (Fig. 1, A and C). Exposure to 10 and 20 ng/ml TNF-α resulted in significant augmentation of CD38 mRNA expression and ADP-ribosyl cyclase activity in ASMNA and ASMA cells (n = 8). However, the augmented CD38 expression and ADP-ribosyl cyclase activity were higher in ASMA than ASMNA cells at 10 and 20 ng/ml TNF-α. ADP-ribosyl cyclase activity was consistently lower after exposure to 40 ng/ml TNF-α than at the lower TNF-α concentrations in ASMA and ASMNA cells (Fig. 1C). There was no significant cytotoxicity over the range of TNF-α concentrations used in the present study (data not shown). In subsequent studies, 10 ng/ml TNF-α was used. TNF-α-induced CD38 mRNA expression showed a time-dependent increase in ASMNA and ASMA cells, with the differential elevation of CD38 mRNA expression starting to appear at 6 h after TNF-α exposure (n = 3; Fig. 1B). Another enzyme activity of CD38, cADPR hydrolase activity, was also differentially elevated in ASMA cells compared with ASMNA cells following exposure to TNF-α (10 ng/ml) for 24 h (n = 3; Fig. 1D). Although a mixture of tracheal and bronchial ASM cells was used, we did not find differences in CD38 mRNA expression or ADP-ribosyl cyclase activity between ASM cells isolated from these locations.

To determine whether the TNF-α-induced CD38 expression is sensitive to inhibition by glucocorticoids, HASM cells were pretreated with 10 nM dexamethasone for 1 h and then with 10 ng/ml TNF-α for 24 h in the continued presence of dexamethasone. Pretreatment of the cells with 10 nM dexamethasone attenuated TNF-α-induced CD38 expression in both groups of HASM cells (n = 3; Fig. 1, E and F). Three of the 9 asthmatic donors in the study had a history of severe asthma. However, there were no quantitative differences in the differential expression of CD38 in the cells from the severely asthmatic donors compared with the myocytes from the rest of the asthmatic donors (n = 3; Fig. 2). Furthermore, the differentially elevated CD38 mRNA expression in ASMA cells was maintained through subsequent passages of the primary cultures (n = 3; Fig. 2). The differential induction of CD38 expression by TNF-α was not due to increased expression of TNF-α receptor in ASMA cells, as TNFR1 levels were comparable between the ASMA and ASMNA cells (data not shown).

**MAPK activation in HASM cells.** We previously showed that the TNF-α-induced CD38 expression is mediated through the MAPKs, ERK 1/2, and JNK (37). To determine whether the differential induction of CD38 in response to TNF-α in the ASMA cells was due to increased activation of the MAPKs, we measured basal and TNF-α-induced activation of the MAPKs. In four of the six ASMA cells, increased levels of phosphorylated ERK MAPK and, to a lesser extent, p38 MAPK were detected in lysates obtained from ASMA cells (n = 6; Fig. 3). After exposure to TNF-α, there was further elevation of activated ERK and p38 MAPKs, with a higher level of ERK activation in the ASMA than ASMNA cells. There was no basal activation of JNK MAPK in ASMA or ASMNA cells (n = 6; Fig. 3, C and F). Moreover, TNF-α-induced JNK activation was consistently higher in ASMNA than ASMA cells (Fig. 3, C and F).

To determine the role of ERK and p38 MAPKs in TNF-α-induced CD38 expression in ASMA cells, ADP-ribosyl cyclase activity was measured in cells treated with TNF-α in the presence of vehicle or the inhibitors of MEK1/2 (U0126) or p38 (SB203580) MAPK. Inhibition of ERK and p38 MAPKs attenuated TNF-α-induced ADP-ribosyl cyclase activity in ASMA and ASMNA cells by >50% (n = 3; Fig. 4A). To determine whether the ASMA cells show differential sensitivity to the pharmacological inhibitors of MAPKs, cells were exposed to various concentrations of each MAPK inhibitor. ERK, p38, and JNK MAPK inhibitors attenuated TNF-α-
Asthma and Non-asthma CD38 expression in airway smooth muscle.

A. Western blot analysis showing CD38 and GAPDH expression in V, T20, and T10 groups.

B. CD38 mRNA expression over time after TNF-α treatment.

C. ADP-ribosyl cyclase activity over time after TNF-α treatment.

D. Net CADPR hydrolyase activity in Non-asthma and Asthma groups.

E. CD38 mRNA expression in C, T, and T+D groups.

F. ADP-ribosyl cyclase activity in C, T, and T+D groups.
induced CD38 mRNA expression comparably in ASMNA and ASMA cells (Fig. 4B; n = 3).

Decay kinetics of CD38 mRNA in HASM cells. In a previous study, we provided evidence for transcript stability in ERK and p38 MAPK regulation of TNF-α-induced expression of CD38 in HASM cells (37). Therefore, we hypothesized that the differential induction of CD38 expression in ASMA cells following exposure to TNF-α results from increased stability of CD38 mRNA. To test this hypothesis, HASM cells were treated with 10 ng/ml rhTNF-α for 12 h and exposed to 5 μg/ml actinomycin D to arrest further transcription, and total RNA was collected at 0, 1, 3, and 12 h. The abundance of CD38 mRNA analyzed by quantitative RT-PCR at each time point showed no significant difference between the ASMNA and ASMA cells (Fig. 5A; n = 3). In another set of studies, HASM cells were incubated in vehicle or TNF-α for 24 h, and TNF-α was removed. Total RNA was collected from the cells 0, 12, and 24 h after removal of TNF-α, and the remaining CD38 mRNA was determined by quantitative RT-PCR. There was no difference in CD38 mRNA content between the ASMNA and ASMA cells following withdrawal of TNF-α (Fig. 5B; n = 3).

Activation of transcription factors in HASM cells. In HASM cells from nonasthmatic donors, regulation of CD38 expression occurs through the transcription factors NF-κB and AP-1 and involves p38 and JNK MAPKs (37, 38). To determine whether increased activation of these transcription factors contributes to the differential expression of CD38 in ASMA cells, we determined nuclear levels of NF-κB and AP-1 subunits in these cells. HASM cells were treated with vehicle or TNF-α (10 ng/ml) for 1 h to obtain nuclear proteins. The nuclear extracts were resolved in SDS-PAGE and immunoblotted to determine levels of the p50 subunit of NF-κB (n = 5) or c-Fos and phosphorylated c-Jun/c-Jun (n = 4) as a measure of AP-1 activation. There was no significant nuclear translocation of NF-κB under basal conditions in either group (Fig. 6A). Exposure to TNF-α caused an increase in NF-κB nuclear translocation in both groups of cells (Fig. 6A). In two of the five preparations, the increase in nuclear NF-κB following TNF-α treatment appeared greater in ASMA than ASMNA cells (Fig. 6A, left), although this increase was not seen in cells from three other asthmatic donors (Fig. 6A, right). ELISA showed that the binding of NF-κB to the consensus NF-κB oligonucleotide was comparable in nuclear lysates from ASMA and ASMNA cells (Fig. 6D). These findings were confirmed by EMSA (Fig. 6F). Irrespective of the magnitude of NF-κB activation, expression of CD38 was greater in all preparations of ASMA cells included in this study than in ASMNA cells following exposure to TNF-α. TNF-α-induced nuclear translocation of c-Fos was lower in ASMA than ASMNA...
cells (Fig. 6B), whereas nuclear translocation of phosphorylated c-Jun was comparable in both groups of cells (Fig. 6C). ELISA-based analysis showed that TNF-α-induced phosphorylated c-Jun binding to its consensus sequence was comparable in ASMNA and ASMA cells (Fig. 6G). Western blot analysis of C/EBPβ in nuclear extracts revealed consistently lower levels of this transcription factor in ASMA than ASMNA cells (Fig. 6B; n = 4).

DISCUSSION

In the present study, we demonstrate that ASM cells obtained from asthmatic donors (donors who died following an episode of asthma or those who had a history of asthma) exhibit increased sensitivity to TNF-α as well as significantly enhanced CD38 expression in response to TNF-α. Also we found differences in the activation of MAPKs in ASM cells obtained from some asthmatic donors. Furthermore, our findings indicate that stability of the CD38 transcript may not account for the differential induction of CD38 expression by the inflammatory cytokine in the ASMA cells. Therefore, we hypothesize that transcriptional regulation largely contributes to the observed differential induction of CD38 expression in ASMA cells by the inflammatory cytokine TNF-α, although this differential induction appears not to be related to altered activation of the transcription factors NF-κB and AP-1.

In the present study, we analyzed the expression of CD38 in response to TNF-α in cells isolated from nine asthmatic donors and found it to be significantly higher than in cells from nonasthmatic donors. Although three of the nine asth-
matic donors died from unstable asthma, there appears to be no clear correlation between disease severity and the response to TNF-α. We also did not find significant constitutive expression of CD38 mRNA in ASMA cells, contrary to our prediction. It is plausible that CD38 signaling in ASM cells contributes to hyperresponsiveness under conditions of active inflammation. Other investigators reported differences between ASM cells from asthmatic and nonasthmatic donors that become evident only after an inflammatory stimulus (26). Furthermore, CD38 expression in ASMA cells in response to TNF-α is sensitive to glucocorticoids, as is the response in ASMNA cells. It will be interesting to examine this in cells obtained from donors with a documented history of steroid-resistant asthma.

We found that the activation status of ERK and p38 MAPKs was variable among the ASMA cells. In four of the six ASM cells isolated from donors with asthma, the levels of phosphorylated ERK and p38 MAPKs appeared greater in ASMA than ASMNA cells under basal conditions, as well as following TNF-α stimulation, although this increased basal activation did not result in elevated CD38 expression. This disconnect between the basal MAPK activation and CD38 expression could be due to 1) subthreshold levels of ERK or p38 activation in the basal state in ASMA cells (compared with the “normal” ASMNA cells) or 2) reduced role of MAPK signaling in CD38 expression in ASMA cells compared with ASMNA cells. In light of the findings that the inhibitors of ERK, p38, or JNK similarly attenuate CD38 expression in ASMA and ASMNA cells, we speculate that the basal ERK or p38 activation in ASMA cells is not sufficient to trigger CD38 expression in these cells. Interestingly, the ASMA cells obtained from two donors with a history of severe asthma did not show elevated ERK or p38 activation, while the differential elevation of CD38 expression was maintained in these cells. Activated JNK MAPK levels were consistently lower in ASMA than ASMNA cells following TNF-α treatment, although the reduced JNK activation in ASMA cells was not reflected in the nuclear levels of phosphorylated c-Jun or in phosphorylated c-Jun binding to the AP-1 consensus sequence. On the other hand, nuclear c-Fos levels following exposure to TNF-α were lower in ASMA than ASMNA cells. Although differentially elevated ERK and p38 activation was previously reported in airways of asthmatic individuals (28) and in the present study, the mechanisms involved in the differential activation are not known. In the present study, we found that the expression of MKP-X (also called Pyst2), a dual-specificity phosphatase that selectively dephosphorylates ERK MAPK, was comparable in ASMNA and ASMA cells (data not shown).
In a previous study, we showed that inhibition of ERK and p38 MAPKs reduced CD38 transcript stability (37). The fact that the stability of CD38 transcripts following TNF-α exposure was similar in ASMA and ASMNA cells suggests that transcriptional regulation may have a larger role in the differential induction of CD38 expression. However, the differentially elevated ADP-ribosyl cyclase and cADPR hydrolase activities in the ASMA cells reflect increased CD38 protein content and suggest translational mechanisms in this differential elevation.

We and others have shown that NF-κB and AP-1 transcription factors have a major role in the regulation of CD38 expression in mammalian cells (36, 38). Mutagenesis of the NF-κB binding site or one of the six AP-1 binding sites of the cd38 gene results in complete loss of TNF-α-induced CD38 promoter activation, suggesting a key role for these elements in transcription (38). Increased nuclear levels and DNA-binding activity of NF-κB have been reported in cells obtained from the sputum and bronchial biopsy of asthmatic patients (18). A recent study reported increased transcriptional activation of CXCL8 promoter in ASMA cells through increased binding of the NF-κB p65 subunit to the promoter without a differential elevation of nuclear p65 (22). Furthermore, evidence from other cell systems indicates that members of the C/EBP family of proteins inhibit NF-κB-mediated transcription (41). A consistent finding of the present study relates to decreased C/EBPβ levels in the nuclear fractions obtained from ASMA cells compared with ASMNA cells under basal conditions, as well as following TNF-α exposure. These observations prompted us to hypothesize that transcriptional regulation through NF-κB may make a larger contribution to the differential elevation of CD38 in ASMA cells. Contrary to our prediction, we found that the binding of NF-κB to the consensus NF-κB sequence, determined by two independent methods, was comparable in nuclear lysates from ASMA and ASMNA cells. These findings suggest that the NF-κB-mediated transcriptional regulation, while critical, may not account for the differential increase in CD38 expression in ASMA cells.

TNF-α has an important role in the pathogenesis of asthma (4, 13, 40). One study reported that the TNF-α signaling axis is upregulated in peripheral blood monocytes from severe, corticosteroid-refractory asthmatic patients, but not in patients with mild-to-moderate asthma (2). In the present study, we found comparable levels of TNFR1 expression in ASMNA and ASMA cells (data not shown), suggesting that the differentially elevated sensitivity of ASMA cells to TNF-α potentially arises from subreceptor signaling cascades. A recent study also reported that the surface levels of the TNF-α receptors TNFR1 and TNFR2 were comparable between ASM cells isolated from asthmatic and nonasthmatic donors (26). We speculate that this differential sensitivity to the inflammatory cytokine is conferred at the level of MAPKs. Our previous investigations in HASM cells found that JNK MAPK mediates TNF-α-induced CD38 expression through transcriptional mechanisms (37). The present finding of a differential elevation of TNF-α-induced CD38 expression in ASMA cells in the presence of reduced JNK activation suggests that the ASMA cells may have been programmed to recruit specific MAPK pathways that are different from those of the normal airway myocytes.

Intrinsic differences between ASM cells obtained from asthmatic and nonasthmatic donors in terms of signaling pathways have been described. These include decreased C/EBPβ, differential recruitment of phosphatidylinositol 3-kinase over the ERK MAPK pathway in the regulation of cell proliferation, and differential elevation of the activities of transcription factors (5, 22, 35). Evidence for phenotypic heterogeneity of ASM cells has been reported, with one subset of smooth muscle cells within the airways expressing higher levels of contractile proteins (17). It is likely that, in asthma, a specific
phenotype of ASM cells predominates and exhibits higher sensitivity to cytokines such as TNF-α. In support of this phenomenon of predominant phenotype, we found that the sensitivity of ASMA cells to TNF-α increased by severalfold in later passages. It has been reported that ASMA cells show elevated proliferative capability (23). We speculate that the highly proliferative cells in the airways of asthma patients may exhibit increased sensitivity to TNF-α.

Fig. 6—Continued.
In summary, in the present study, we describe differential induction in the ASMA cells of CD38 expression by TNF-α (T10), which was not an outcome of differentially elevated activation of NF-κB or AP-1. This differential elevation of CD38 in ASMA cells was associated with reduced activation of JNK MAPK and increased activation of ERK and p38 MAPKs. Since the CD38 mRNA stability may not be a factor in the elevated CD38 expression in cells from asthmatic patients, the role of other transcription factors, increased rate of transcription, and translational regulatory mechanisms should be considered in future studies exploring the mechanisms associated with these findings.

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**DISCLOSURES**
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