Tumor Necrosis Factor-α–Induced Activation of RhoA in Airway Smooth Muscle Cells: Role in the Ca\(^{2+}\) Sensitization of Myosin Light Chain\(_{20}\) Phosphorylation

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ABSTRACT

Tumor necrosis factor-α (TNF), an inflammatory cytokine, has a potentially important role in the pathogenesis of bronchial asthma and may contribute to airway hyper-responsiveness. Recent evidence has revealed that TNF can increase the Ca\(^{2+}\) sensitivity of agonist-stimulated myosin light chain\(_{20}\) (MLC\(_{20}\)) phosphorylation and contractility in guinea pig airway smooth muscle (ASM). In the present study, the potential intracellular pathways responsible for this TNF-induced Ca\(^{2+}\) sensitization were investigated. In permeabilized cultured guinea pig ASM cells, recombinant human TNF stimulated an increase in Ca\(^{2+}\)-activated MLC\(_{20}\) phosphorylation under Ca\(^{2+}\) “clamp” conditions. This increased MLC\(_{20}\) phosphorylation was inhibited by preincubation with the Rho-kinase inhibitor Y27632. TNF also increased the proportion of GTP-bound RhoA, as measured using rhotekin Rho-binding domain, in a time course compatible with a role in the TNF-induced Ca\(^{2+}\) sensitization. In cultured human ASM cells, recombinant human TNF also activated RhoA with a similar time course. In addition, TNF stimulated phosphorylation of the regulatory subunit of the myosin phosphatase, which was inhibited by Y27632. Although human ASM cells expressed both receptor subtypes, TNF-R1 and TNF-R2, the activation of RhoA was predominantly via stimulation of the TNF-R1, although RhoA did not immunoprecipitate with the TNF-R1. In conclusion, the TNF-induced increase in the Ca\(^{2+}\) sensitivity of MLC\(_{20}\) phosphorylation is through stimulation of the TNF-R1 receptor and via a RhoA/Rho-kinase pathway leading to inhibition of the myosin light chain phosphatase. This intracellular mechanism may contribute to TNF-induced airway hyper-responsiveness.

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ABBREVIATIONS: TNF, tumor necrosis factor-α; MLC\(_{20}\), myosin light chain\(_{20}\); GEF, guanine exchange factor; TNF-R, tumor necrosis factor-α receptor; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; PIPES, 1,4-piperazinediethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; RBD, Rho-binding domain; GTP\(_{S}\), guanosine S’-O-(3-thio)triphosphate; TRAF, tumor necrosis factor-α receptor-associating factor; Y27632, (R)-(−)-trans-N-(4-pyridyl)-4-(L-aminoethyl)-cyclohexane carboxamide.
exchange factors (GEFs) (Hart et al., 1998; Koza449s et al., 1998). Activation of the monomeric G protein RhoA by seven-transmembrane G protein-coupled receptors may be of particular importance in many smooth muscle types (Somlyo and Somlyo, 2000). Activation of RhoA leads to subsequent activation of a recently isolated downstream target of Rho, p160 Rho-associated coiled-coil-containing protein kinase (Rho-kinase) (Leung et al., 1995; Matsui et al., 1996). Rho-kinase directly phosphorylates the regulatory subunit (MYPT-1) of the smooth muscle myosin light chain phosphatase (Feng et al., 1999), either directly or via an additional myosin phosphatase-associated kinase (Borman et al., 2002). This phosphorylation results in an inhibition of phosphatase activity leading to increased accumulation of phosphorylated MLC_{20} (Kimura et al., 1996) and subsequently an increased Ca^{2+} sensitivity to contraction. We have recently uncovered the first evidence of the cellular mechanism whereby TNF pretreatment of bronchial smooth muscle directly increases maintained agonist-induced responses (Parris et al., 1999). It is now clear that TNF, although not a Ca^{2+}-releasing agonist, potentiates the Ca^{2+}-activated contractile response via an increase in MLC_{20} phosphorylation. This TNF-induced increase in the Ca^{2+} sensitivity of MLC_{20} phosphorylation is the result of TNF receptor activation and intracellular signal transduction pathways that ultimately regulate smooth muscle contractility. TNF is known to activate a variety of signaling cascades in airway smooth muscle (Emala et al., 1997; Amrani et al., 2000b, 2001; McFarlane et al., 2001). Because there is no experimental evidence to suggest that TNF receptor subtypes (TNF-R1 and TNF-R2) can couple to heterotrimeric G proteins, the activation of these Ca^{2+}-sensitizing pathways is likely to occur via as yet undescribed signaling cascades, possibly involving either a potentiation of myosin light chain kinase activity or inhibition of the myosin light chain phosphatase activity.

In this study, we show that the TNF-induced Ca^{2+} sensitization of MLC_{20} phosphorylation in ASM cells from both guinea pig and human is regulated by activation of RhoA in a time course compatible with the TNF-induced airway hyper-responsiveness. This is predominantly via the TNF-R1 receptor. This Ca^{2+} sensitization is induced by an inhibition of the smooth muscle myosin light chain phosphatase activity, probably via Rho-kinase activation.

**Materials and Methods**

**Reagents.** Recombinant human TNF-α was purchased from R & D Systems (Abingdon, UK). Mutated forms ("muteins") of human TNF-α, which allow selective activation of either TNF-R1 (R32W, S867/T1-R-TNF) or TNF-R2 (D143N, A145R/R2-TNF), have been described previously (Van Ostade et al., 1994). Monoclonal antibodies against RhoA (26C4) and TNF-R1 (H5) and a polyclonal antibody against TNF-R2 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Polyclonal antibodies against MYPT-1 and phospho-MYPT-1 (Thr686) were from Upstate Biotechnology (Lake Placid, NY). The Rho-kinase inhibitor Y27632 was purchased from Rho-kinase Overcome the first evidence of the cellular mechanism whereby TNF pretreatment of bronchial smooth muscle directly increases maintained agonist-induced responses (Parris et al., 1999). It is now clear that TNF, although not a Ca^{2+}-releasing agonist, potentiates the Ca^{2+}-activated contractile response via an increase in MLC_{20} phosphorylation. This TNF-induced increase in the Ca^{2+} sensitivity of MLC_{20} phosphorylation is the result of TNF receptor activation and intracellular signal transduction pathways that ultimately regulate smooth muscle contractility. TNF is known to activate a variety of signaling cascades in airway smooth muscle (Emala et al., 1997; Amrani et al., 2000b, 2001; McFarlane et al., 2001). Because there is no experimental evidence to suggest that TNF receptor subtypes (TNF-R1 and TNF-R2) can couple to heterotrimeric G proteins, the activation of these Ca^{2+}-sensitizing pathways is likely to occur via as yet undescribed signaling cascades, possibly involving either a potentiation of myosin light chain kinase activity or inhibition of the myosin light chain phosphatase activity.

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**Cell Isolation and Culture.** Male Duncan Hartley guinea pigs were killed by cervical dislocation, and the trachea was removed and placed in Hanks' balanced salt solution. After removal of fat and connective tissue, the smooth muscle was cut into small pieces and incubated in serum-free DMEM containing 2 mM L-glutamine, penicillin (10,000 units/ml), and streptomycin (10 mg/ml); and incubated at 37°C in a humidified 5% CO_2 atmosphere. After 24 h, the medium was removed and cells were transferred to fresh DMEM, 10% FBS, 2 mM L-glutamine, penicillin (10,000 units/ml), and streptomycin (10 mg/ml); and incubated at 37°C in a humidified 5% CO_2 atmosphere. After 24 h, the medium was removed and cells were transferred to fresh DMEM, 10% FBS, 2 mM L-glutamine, penicillin (10,000 units/ml), and streptomycin (10 mg/ml); and incubated at 37°C in a humidified 5% CO_2 atmosphere. After 24 h, the medium was removed and cells were transferred to fresh DMEM, 10% FBS, 2 mM L-glutamine, penicillin (10,000 units/ml), and streptomycin (10 mg/ml); and incubated at 37°C in a humidified 5% CO_2 atmosphere. After 24 h, the medium was removed and cells were transferred to fresh DMEM, 10% FBS, 2 mM L-glutamine, penicillin (10,000 units/ml), and streptomycin (10 mg/ml); and incubated at 37°C in a humidified 5% CO_2 atmosphere. After 24 h, the medium was removed and cells were transferred to fresh DMEM, 10% FBS, 2 mM L-glutamine, penicillin (10,000 units/ml), and streptomycin (10 mg/ml); and incubated at 37°C in a humidified 5% CO_2 atmosphere.
mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% Triton X-100, 1 mM EDTA, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 10 μg/ml pepstatin and clarified by centrifugation at 15,000g for 15 min at 4°C. Supernatants were incubated with either control IgG, anti-RhoA, or anti-TNF-R1 antibody. Immune complexes were collected by incubation with protein G-Sepharose beads, washed three times with lysis buffer, boiled in SDS sample buffer, and analyzed by immunoblotting. Control experiments demonstrated that, under these conditions, the anti-TNF-R1 antibody can coprecipitate proteins normally associated with TNF-R1.

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting. Cell lysates were mixed with an equal volume of 2× SDS sample buffer and incubated at 100°C for 5 min. Lysates were fractionated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. After blocking with 5% nonfat milk powder in Tris-buffered saline, pH 7.4, containing 0.1% Tween 20 for 1 h at RT, blots were incubated with primary antibody for 1 h at RT or overnight at 4°C, washed, and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at RT. Immunoreactive species were visualized using enhanced chemiluminescence and quantitated by scanning densitometry using a GS-690 imaging densitometer (Bio-Rad, Hercules, CA).

Results

TNF-Induced Increase in Ca2+-Activated MLC20 Phosphorylation in Cultured Guinea Pig ASM Cells. We have previously demonstrated that TNF treatment of permeabilized guinea pig bronchial smooth muscle strips results in Ca2+ sensitization of the myofilaments and a corresponding increase in MLC20 phosphorylation (Parris et al., 1999). In attempting to dissect the underlying mechanism of this novel signaling pathway, we have examined the ability of TNF to stimulate MLC20 in permeabilized cultured guinea pig ASM. In initial studies, the effect of GTPγS, a nonhydrolysable analog of GTP, known to produce an increase in the Ca2+ sensitivity of MLC20 phosphorylation in permeabilized smooth muscle (Fu et al., 1998), was determined. After a 2-min incubation in pCa 6.8 with 100 μM GTPγS, the MLC20 phosphorylation was significantly increased compared with pCa 6.8 alone (Fig. 1). Because GTPγS is membrane-impermeant, these results demonstrate that the cells are effectively permeabilized and that the intracellular signaling pathways are intact.

To determine the ability of TNF to increase the Ca2+ sensitization of MLC20 phosphorylation, permeabilized cultured guinea pig ASM cells were incubated with 200 ng/ml recombinant human TNF for 45 min and exposed to pCa 6.8 for 2 min. TNF incubation produced a significant increase in Ca2+-activated MLC20 phosphorylation compared with controls incubated in the absence of TNF (Fig. 1). Controls incubated with TNF but not stimulated with pCa 6.8 also showed no increase MLC20 phosphorylation (data not shown). This increase in MLC20 phosphorylation stimulated by TNF, is similar to that observed in TNF-treated guinea pig bronchial smooth muscle (Parris et al., 1999), indicating that cultured ASM cells represent a suitable model system in which to investigate the molecular mechanism of TNF-induced Ca2+ sensitization.

Involvement of the RhoA/Rho-Kinase Pathway in the TNF-Induced Ca2+ Sensitization of Cultured Guinea Pig ASM Cells. In smooth muscle, Ca2+ sensitization by a number of agonists has been shown to be dependent on the small G protein RhoA (Gong et al., 1997) and its downstream effector Rho-kinase. To examine the role of the RhoA/Rho-kinase pathway in TNF-induced MLC20 phosphorylation, permeabilized guinea pig ASM cells were treated with the Rho-kinase inhibitor Y27632, before incubation with 200 ng/ml TNF and subsequent stimulation with pCa 6.8 buffer as described above. The TNF-induced increase in the Ca2+-activated MLC20 phosphorylation was significantly inhibited by preincubation with Y27632 (Fig. 2A). Y27632

![Fig. 1. Effect of TNF and GTPγS on Ca2+-activated MLC20 phosphorylation in permeabilized guinea pig ASM cells. A, typical colloidal gold-stained membranes showing nonphosphorylated (NP), monophosphorylated (P1), and diphosphorylated (P2) MLC20. B, graph showing mean MLC20 phosphorylation expressed as a percentage of the total MLC20. Data are mean ± S.E.M. n = 8 for TNF-treated samples, and n = 5 for GTPγS-treated cells.](attachment:image)
alone had no significant effect on Ca\textsuperscript{2+}-activated MLC\textsubscript{20} phosphorylation in the absence of TNF.

To determine whether TNF can directly activate RhoA in cultured guinea pig ASM cells, a GST-rhotekin RBD pull-down assay was used (Ren et al., 1999). A detailed time course revealed that TNF caused a rapid and potent activation of RhoA in cultured guinea pig ASM cells (Fig. 2B). The level of GTP-RhoA was increased within 1 min, reaching peak activation of 3.5-fold within 15 min. The level of activated RhoA decreased sharply at 30 min, returning close to basal levels by 60 min. Taken together, these results support the notion that TNF-stimulated MLC\textsubscript{20} phosphorylation is dependent on the RhoA/Rho-kinase pathway.

### Involvement of the RhoA/Rho-Kinase Pathway in TNF-Induced Ca\textsuperscript{2+} Sensitization of Human Cultured ASM Cells.

Because human TNF may have an altered affinity and/or efficacy when bound to guinea pig TNF receptors, the effects of human TNF on primary cultured human ASM cells were also investigated. In human cells, as in guinea pig ASM cells, TNF induced an increase in GTP-bound RhoA as assessed by the GST-rhotekin RBD pull-down assay (Fig. 3A). RhoA was activated more rapidly in human than guinea pig ASM cells, with a 2-fold increase in the level of GTP-RhoA detected within 1 min. Maximal RhoA activation (4-fold) was reached within 5 min, and thereafter activation declined, returning to basal levels by 60 min.

To determine potential involvement of TNF-induced RhoA activation of Ca\textsuperscript{2+} sensitization in human ASM cells, two-dimensional gel electrophoresis was carried out to determine the extent of MLC\textsubscript{20} phosphorylation. In human ASM cells, a separation of MLC\textsubscript{20} isoforms by two-dimensional gel electrophoresis revealed a complex pattern of spots at the pl and molecular weight of MLC\textsubscript{20} (data not shown). These multiple spots were also recognized in immunoblots with a specific anti-MLC antibody. This suggests multiple isoforms and possibly additional phosphorylation states. TNF-induced Ca\textsuperscript{2+} sensitization of MLC\textsubscript{20} phosphorylation in human ASM cells could therefore not be assessed further using this method.

As an alternative, we examined the effect of TNF on the phosphorylation of MYPT-1, the regulatory subunit of myosin phosphatase. Phosphorylation of MYPT-1 inhibits phosphatase activity and leads to an increase in MLC\textsubscript{20} phosphorylation (Feng et al., 1999). Treatment with TNF resulted in a 2-fold increase in MYPT-1 phosphorylation within 1 min and reached a maximal 4.7-fold activation within 5 min (Fig. 3B). The level of phosphorylated MYPT-1 decreased slightly by 15 min but remained elevated even after 60 min. TNF-induced MYPT-1 phosphorylation was completely abrogated by pretreatment of cells with the Rho-kinase inhibitor Y27632 (Fig. 3C). These data demonstrate that in human ASM cells, TNF-induced activation of the RhoA/Rho-kinase pathway results in phosphorylation of MYPT-1.

### TNF Receptor Subtypes Involved in the TNF-Induced Activation of RhoA.

The effects of TNF are mediated by two receptors, TNF-R1 and TNF-R2 (MacEwan, 2002). To examine the expression of these receptors in cultured ASM cells, cell extracts were analyzed by immunoblotting with antibodies specific for the receptor subtypes. Hela cells, which have a previously defined expression of TNF-R1 and TNF-R2 (Grell et al., 1998), were used as a comparison. Both TNF-R1 and TNF-R2 were detected in human ASM cells and had apparent molecular weights identical to those in Hela cells (Fig. 4A). Human ASM cells contained approximately 3-fold less TNF-R1 and 1.5-fold more TNF-R2, compared with Hela cells. As previously published, Hela cells...
have a TNF receptor subtype ratio of approximately 95% TNF-R1:5% TNF-R2 (Grell et al., 1998).

To determine the role of TNF receptor subtypes in the activation of the RhoA/Rho-kinase pathway, we have used muteins of TNF, which selectively bind to and activate either TNF-R1 or TNF-R2. Because these muteins are derived from the human TNF sequence and may therefore not be selective for guinea pig receptors, their effect was assessed on human ASM cells only. The TNF-R1 mutein stimulated an increase in GTP-RhoA in human ASM cells (Fig. 4B) with a time course identical to that observed for recombinant human TNF (Fig. 3A), although the magnitude of the increase was greater with the TNF-R1 mutein (6-fold increase compared with 4-fold increase for recombinant human TNF). The TNF-R2 mutein also stimulated an increase in GTP bound RhoA (Fig. 4C); however, this level was significantly lower than that stimulated by either the TNF-R1 mutein or recombinant TNF (peak increase 2-fold).

To determine whether TNF-R1 can interact with RhoA, extracts of human ASM cells, treated without or with TNF, were immunoprecipitated with anti-TNF-R1 receptor antibody. Subsequent immunoblotting failed to reveal coprecipitation of RhoA, although RhoA was effectively immunoprecipitated with the RhoA antibody (Fig. 5). We were also unable to detect any interaction between RhoA and the TNF-R1 adaptor proteins TNF receptor-associating factor 1 (TRAF1) and TNF receptor-associated factor 2 (TRAF2).
(TRAF)-2, receptor interacting protein, and TNF receptor-associated death domain binding protein (data not shown). In positive control experiments, RhoA was capable of coprecipitating with Rho-kinase.

**Discussion**

There is considerable in vivo evidence of an important role for TNF in airway hyper-responsiveness (Kips et al., 1992; Thomas et al., 1995; Renzetti et al., 1996). The present study describes a molecular mechanism that may be, at least in part, responsible for producing acute airway hyper-responsiveness (Thomas et al., 1995). TNF can increase the Ca²⁺ sensitivity of MLC₂₀ phosphorylation in ASM cells, from both guinea pigs and humans, via a Rho-kinase-dependent pathway. In addition, TNF can activate RhoA in a time course compatible with a role in airway hyper-responsiveness and the TNF-induced potentiation of contractility previously observed in guinea pig bronchial smooth muscle (Parris et al., 1999). This activation is predominantly via TNF-R1.

Previous studies on the intracellular effects of TNF in ASM cells have concentrated predominantly on elucidating the mechanisms involved in longer term effects resulting in alterations of protein expression (e.g., heterotrimeric GTP-binding proteins) (Hotta et al., 2000) and intracellular adhesion molecule-1 (Amrani et al., 2000b, 2001). These TNF-induced effects are likely to be of importance in chronic inflammatory responses and possibly ASM cell proliferation; however, they do not address the potential mechanisms of acute airway hyper-responsiveness induced by TNF in a much shorter time course (Kips et al., 1992; Thomas et al., 1995). Our previous study suggested such a mechanism may occur via a TNF-induced increase in the Ca²⁺ sensitivity of ASM contraction (Parris et al., 1999), although TNF itself is not a contractile agonist and does not increase intracellular Ca²⁺ in ASM cells; i.e., TNF is acting only as a sensitizing agent. In permeabilized guinea pig bronchial smooth muscle strips, TNF incubation for 45 min significantly increased the Ca²⁺-activated contractility via an increase in the Ca²⁺-activated MLC₂₀ phosphorylation. The increase in the MLC₂₀ phosphorylation in guinea pig ASM cells in the present study was inhibited by preincubation with Y27632, a Rho-kinase inhibitor. Rho-kinase is known to play an important role in regulating contractility of airway (Iizuki et al., 1999, 2000; Ito et al., 2001) and other types of smooth muscle (Kureishi et al., 1997; Fu et al., 1998). To date, this regulation has been associated with contractile agonists activating seven-transmembrane receptors and heterotrimeric G proteins (Somlyo and Somlyo, 2000). This study now demonstrates a role for TNF, a noncontractile agonist, in regulating ASM contractility via activation of Rho-kinase. Further evidence for this mechanism is provided by TNF-induced activation of RhoA in both guinea pig and human ASM cells. The slower time of peak Rho activation in guinea pig ASM cells may represent a decreased relative efficacy and/or affinity of recombinant human TNF on guinea pig TNF receptors. However, this may also represent physiological differences in the TNF-induced Ca²⁺ sensitization in guinea pig versus humans. The fold levels of increase in GTP-bound RhoA observed here are similar in magnitude to those observed in other smooth muscles stimulated with contractile agonists such as U46619, norepinephrine, and endothelin-1 (Sakurada et al., 2001).

Although our initial study first revealed the TNF-induced increase in Ca²⁺ sensitivity of MLC₂₀ phosphorylation in guinea pig ASM, it is important to determine whether this effect occurs in human. TNF does produce an in vivo airway hyper-responsiveness in humans (Thomas et al., 1995). In cultured human ASM cells, it was not possible to determine TNF-induced MLC₂₀ phosphorylation using two-dimensional gel electrophoresis until the apparently multiple isoforms of MLC₂₀ have been further characterized. Phosphorylation of the MYPT-1 from the smooth muscle myosin light chain phosphatase, however, was assessed as an indicator of Ca²⁺ sensitization. TNF incubation increased phosphorylation of MYPT-1 in a time course compatible with TNF-induced Ca²⁺ sensitization; however, the phosphorylation levels remained elevated for a longer time period and were above control levels even after 1 h. This is in agreement with a recent study showing that dephosphorylation of MYPT-1 is relatively slow (Takizawa et al., 2002) and consistent with the prolonged Ca²⁺ sensitization observed after TNF incubation (Parris et al., 1999). Phosphorylation of MYPT-1 was blocked by the Rho-kinase inhibitor Y27632, suggesting that it is the result of Rho-kinase activation. This study therefore suggests that TNF induces activation of the RhoA/Rho-kinase pathway, leading to phosphorylation and inhibition of the smooth muscle myosin light chain phosphatase and a subsequent increase in the Ca²⁺ sensitivity of MLC₂₀ phosphorylation.

TNF effects are mediated by two receptor subtypes, TNF-R1 and TNF-R2 (MacEwan, 2002). Both TNF-R1 and TNF-R2 receptors are single transmembrane glycoproteins with 28% sequence homology and a marked delineation of function (Grell et al., 1994). As shown in this study by immunoblotting, both these receptor subtypes are present in cultured human ASM cells (Amrani et al., 2000b) and have been previously identified by us in guinea pig ASM cells (McFarlane et al., 2001). TNF receptor engagement activates a variety of signaling molecules. This study is the first to report activation of RhoA by TNF in ASM cells, predominantly via activation of the TNF-R1, with a smaller proportion via the TNF-R2. The intracellular mechanism by which TNF activates RhoA in these cells is not clear. TNF receptors activate downstream signaling pathways via interaction with a family of adaptor proteins (MacEwan, 2002). These proteins, known as TRAFs, bind to the cytoplasmic domain of TNF receptors (Wajant et al., 1999). To date, no direct interaction of TRAFs with either Rho or one of the several GEFs,
which mediate Rho activation, has been identified. TNF-induced RhoA activation has previously been implicated in cytoskeletal reorganization in serum-starved endothelial cells and fibroblasts over a relatively slow time course (approximately 30 min) (Wojciak-Stothard et al., 1998; Puls et al., 1999). This is possibly via a hierarchical cascade of structurally related monomeric G proteins (Puls et al., 1999). This study has directly measured the time course of TNF-induced RhoA activation and this reveals a rapid increase (within 1 min). This fast time course indicates that a directly signaling pathway is probably involved. A direct interaction of RhoA has been previously observed with the neurotrophin receptor p75 (Yamashita et al., 1999), a related member of the TNF receptor family. We investigated whether this also be the case for the TNF-R1 receptor. Under the conditions used in this study no interaction was observed between RhoA and TNF-R1, or the principal TNF-R1 adaptor proteins TRAF-2, receptor interacting protein and TNF receptor-associated death domain binding protein. A recent study in guinea pig ASM cells has suggested that the TNF-induced increase in MLC\(_{20}\) phosphorylation may be via formation of reactive oxygen species (Thabut et al., 2002), although the mechanism linking reactive oxygen species generation to increased MLC\(_{20}\) phosphorylation is not known. It is interesting to note that some effects of reactive oxygen species in the lung occur via Rho-mediated effects (Chiba et al., 2001). It is likely that the TNF receptor, predominantly TNF-R1 (or associated TRAFs), may activate one or more GEFs involved in Rho activation, although this remains to be established.

In conclusion, this study clearly demonstrates that TNF can activate RhoA in ASM cells. In addition, we have also shown that activation of the RhoA/Rho-kinase pathway contributes significantly to the TNF-induced Ca\(_{2+}\)\^{2}\textsuperscript{+} sensitization of MLC\(_{20}\) phosphorylation. These effects are not species-specific and were observed in both guinea pig and human ASM cells. This TNF-activated intracellular mechanism may therefore contribute to the airway hyperresponsiveness observed in inflammatory diseases such as asthma.

References


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