

The Membrane Protein of SARS-CoV Suppresses NF- κ B Activation

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Severe acute respiratory syndrome coronavirus (SARS-CoV) infects many organs, such as lung, liver, and immune organs and causes life-threatening atypical pneumonia, SARS causes high morbidity and mortality rates. The molecular mechanism of SARS pathogenesis remains elusive. Inflammatory stimuli can activate I κ B kinase (IKK) signalsome and subsequently the nuclear factor kappa B (NF- κ B), which influences gene expression of cyclooxygenase-2 (Cox-2) along with other transcription factors. In this work, we found that the membrane (M) protein of SARS-CoV physically interacted with IKK β using a co-immunoprecipitation assay (IPA). Expression of M suppressed tumor necrosis factor alpha (TNF- α) induced NF- κ B activation using a luciferase reporter assay. Further investigation showed M protein suppressed Cox-2 expression using a luciferase reporter gene assay, RT-PCR and Western blot analysis. The carboxyl terminal of M protein was sufficient for the M protein function. Together, these results indicate that SARS-CoV M suppresses NF- κ B activity probably through a direct interaction with IKK β , resulting in lower Cox-2 expression. Suppression of NF- κ B activity and Cox-2 expression may contribute to SARS pathogenesis. *J. Med. Virol.* 79:1431–1439, 2007. © 2007 Wiley-Liss, Inc.

KEY WORDS: SARS-CoV; membrane protein; NF- κ B; Cox-2; IKK β

INTRODUCTION

Severe acute respiratory syndrome (SARS), also called atypical pneumonia, is a severe human respiratory infectious disease that emerged recently in Asia, North America, and Europe. A novel coronavirus named SARS-associated coronavirus (SARS-CoV) has been recently identified as the causative agent of SARS [Drosten et al., 2003; Ksiazek et al., 2003; Peiris et al., 2003]. Morbidity and mortality rate is up to 10% in the

SARS-CoV infected patients [Lee et al., 2003]. The SARS-CoV is an enveloped, positive-stranded RNA virus with a genome comprising approximately 29,727 nucleotides predicted to contain 14 open reading frames (ORFs). A sequence comparison with corresponding ORFs of other known coronaviruses has revealed a pattern of gene organization similar to typical coronaviruses.

The membrane (M) protein of SARS-CoV consists of 221 amino acid residues and the amino acid sequence has low homology to the M proteins of other coronaviruses. The M glycoprotein is among the most abundant viral proteins, spanning the M bilayer three times, with a long carboxyl-terminal cytoplasmic domain inside the virion and short amino-terminal domain outside [Locker et al., 1992; Holmes, 2003]. For other coronaviruses, the M protein is a key player in the assembly of virions at intracellular Ms between endoplasmic reticulum and the Golgi complex. The SARS-CoV M protein can interact with nucleocapsid (N) protein in vitro and in vivo [He et al., 2004; Fang et al., 2005]. It has been reported that the M protein contains highly conserved glycosylated sequences, and its glycosylation may be important for the viral-host interaction [de Haan et al., 1998, 2000].

Nuclear factor kappa B (NF- κ B) transcription factors, as a homo- or heterodimer of c-Rel, RelA (p65), RelB, p50, and p52, play an important role in the regulation of immune and inflammatory responses. Inactivated NF- κ B resides in the cytoplasm by forming complexes with I κ B [Ghosh et al., 1998]. Proinflammatory stimuli, such as tumor necrosis factor alpha (TNF- α) and lipopolysaccharide (LPS), induce signal cascades through their cognate receptors, TNFR and Toll-like receptor

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4 (TLR4), to activate I κ B kinase (IKK) signalsome and subsequently NF- κ B, which plays an essential role in innate and adaptive immune responses to pathogens [Kontgen et al., 1995; Caamano and Hunter, 2002]. IKK signalsome is composed of at least two kinases, IKK α and IKK β , and a regulatory factor, IKK γ . Among these proteins, IKK β is known as a major kinase and IKK γ is required for the full activation of IKK β upon proinflammatory stimulation [Li et al., 1999; Rudolph et al., 2000; Sadikot et al., 2003]. Activated IKK signalsome phosphorylates I κ B, which subsequently undergoes ubiquitination and degradation, exposing nuclear localization signal to allow NF- κ B translocate into the nucleus, which, in turn, activates target genes [Karin and Ben-Neriah, 2000], such as Cox-2, along with other transcription factors, including cyclic-AMP response element (CRE) and CCAAT/enhancer-binding protein (C/EBP) [Hwang et al., 1997; Caivano et al., 2001]. Accordingly, proinflammatory stimuli induce the expression of Cox-2 to catalyze the production of prostaglandins, which promote inflammation through a variety of mechanisms.

There are three known isoforms of cyclooxygenase: Cox-1, which is constitutively expressed; Cox-2, which is expressed in response to inflammatory or mitogenic stimuli; and Cox-3, which is a constitutively expressed splice variant of Cox-1 present in neural tissue [Shaftel et al., 2003]. The Cox-2 gene is 8 kb, containing 10 exons located on chromosome 1, and its promoter contains a TATA box as well as binding sites for a number of transcription factors [Appleby et al., 1994]. Cox-2 shares ~60% of its protein sequence with Cox-1 [Hinz and Brune, 2002]. Both Cox-1 and Cox-2 contribute to homeostasis and to inflammatory pathways [Turini and DuBois, 2002; Parente and Perretti, 2003]. Drugs that preferentially inhibit Cox-1 or both of these enzymes have been used as anti-inflammatory agents.

In this study, we tested whether the SARS-CoV M protein affects NF- κ B activity and Cox-2 production in mammalian cells. Our data demonstrated the SARS-CoV M protein physically interacted with IKK β , suppressed I κ B α protein degradation and NF- κ B dependent

Cox-2 expression. SARS-CoV may evade innate immunity by altering gene expression of key inflammatory molecules.

MATERIALS AND METHODS

Plasmid Construction

The cDNA of the full-length SARS M protein and a truncation mutant M90 (aa 90–221) were amplified by PCR from pGEM[®]-T-M (provided by Dr. Ying Zhu, Wuhan University) using the primers in Table I. The target DNA fragments were cloned into the eukaryotic expression vector pcDNA3.1(-) under the immediately early CMV promoter by using standard cloning methods, to generate pcDNA3.1(-)-M or pcDNA3.1(-)-M90. Plasmids were confirmed with restriction enzyme digestion and sequencing.

Cell Culture and Transfection

Vero E6 (African green monkey kidney cells) and HeLa (Human cervical cancer cells) cells were obtained from China Center for Type Culture Collection (Wuhan, China) and maintained at 37°C with 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA), supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT) plus 100 IU of penicillin and 100 μ g of streptomycin per milliliter. DNA transfection was performed using Lipofectamine[™] 2000 (Invitrogen) according to the manufacturer's instructions.

TNF- α Treatment and Luciferase Assay

Approximately 5×10^4 Vero E6 or HeLa cells were plated onto a 24-well tissue culture plate, and 24 hr later the cells were transfected with luciferase reporter vectors pNF- κ B-Luc or pGL3 COX-2-Luc (provide by Dr. Ying Zhu, Wuhan University) and pcDNA3.1(-)-M or pcDNA3.1(-)-M90 along with *Renilla* luciferase reporter *tk-Renilla-Luc* as an internal control for transfection efficiency. The total amount of transfected plasmids were maintained constant at 0.8 μ g

TABLE I. Gene Specific Custom Primer Sequences

Primer	Sequence
SARS-Cov M protein	
Sense	5'-TAT CGC <u>GCT AGCATG</u> GCA GAC AAC GGT AC-3'
Antisense	5'-TCG GCC <u>GGA TCCCTTA</u> CTG TAC TAG CAA AG-3'
SARS-Cov M90 protein	
Sense	5'-AGT GCG <u>CTA GCA</u> TGG GGC TTA GCT ACT TCG TTG CTT CC-3'
Antisense	5'-TCG GCC <u>GGA TCCCTTA</u> CTG TAC TAG CAA AG-3'
COX-2	
Sense	5'-AGA GGC TAG TGC CTC AGA GAG AA-3'
Antisense	5'-TCG CAT ACA CAA CCC AAA TTC CC-3'
GAPDH	
Sense	5'-ATC ACT GCC ACC CAG AAG AC-3'
Antisense	5'-AGG ATG AGC TGC CCT ATG ATG A-3'

The restriction enzyme sites were underlined. It was *NheI* in the sense primers and *EcoRI* in the antisense primers.

using the pcDNA3.1(-) vector. At 24 hr after transfection, the transfected cells were lysed and assayed for luciferase activity using the Dual-luciferase Reporter Assay system kit (Promega, Madison, WI) following the protocol from the manufacturer. For the TNF- α treatment, at 24 hr post-transfection, cells were treated with TNF- α (10 ng/ml in phosphate-buffered saline (PBS); Sigma) for 6 hr to induce NF- κ B activation and subsequently assayed by luciferase assay. Each experiment was repeated at least three times and data are shown as the average \pm SEM.

Western Blot Assay

Vero E6 or HeLa cells were transfected with pcDNA3.1(-)-M or pcDNA3.1(-)-M90 or pcDNA3.1(-). At 24 hr post-transfection, the cells were washed with cold PBS and lysed in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride, 50 mM NaF, 1 mM sodium orthovanadate, and 0.06 mg/ml aprotinin) on ice for 30 min. After centrifugation at 12,000 rpm for 15 min, the supernatants were separated and protein concentrations were determined by the Bradford assay (Bio-Rad, Hercules, CA). Proteins were separated by 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to nitrocellulose Ms. The Ms were probed with rabbit anti-COX-2 (provided by Dr. Ying Zhu, Wuhan University), anti-M polyclonal antibody (prepared in this study), or mouse monoclonal anti- β -actin, anti-p65, anti-p50, anti-I κ B α , anti-YY1 antibody (Santa Cruz, Santa Cruz, CA), followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG (Santa Cruz). Immunoreactivity was visualized by chemiluminescent detection (Pierce, Rockford, IL) according to manufacturer's protocol.

Reverse Transcription (RT)-PCR

Vero E6 cells were transfected with pcDNA3.1(-)-M or pcDNA3.1(-), and total RNA was extracted from transfected cells by using Trizol reagent (Invitrogen) according to the manufacturer's protocol at 24 hr post-transfection. The cDNA was reverse transcribed from 1.0 μ g total RNA with random hexamer primers using an AMV-reverse transcriptase kit (TaKaRa) as recommended by the supplier. Subsequently, the cDNA preparations were used as the templates for PCR to amplify Cox-2 and GAPDH genes using the primers indicated in Table I. PCR condition was: denaturation at 94°C, annealing at 56°C for GAPDH and Cox-2 primers, and extension at 72°C for 25 cycles using Platinum Taq (Invitrogen).

Preparation of Cytoplasmic and Nuclear Fractions

Vero E6 cells were transfected with pcDNA3.1(-)-M or pcDNA3.1(-). At 24 hr post-transfection, the cells were washed three times with ice-cold PBS and added to

hypotonic buffer (10 mM HEPES [pH 7.9], 5 mM KCl, 1.5 mM MgCl₂, 1 mM NaF, and 1 mM Na₃VO₄), supplemented with dithiothreitol (1 mM), phenylmethylsulfonyl fluoride (1 mM), aprotinin (2 μ g/ml), leupeptin (2 μ g/ml), and soybean trypsin inhibitor (37.5 μ g/ml). After incubation for 15 min on ice, the cytoplasmic fraction was prepared by centrifugation at 3,000 rpm for 5 min and it was cleared by centrifugation at 13,000 rpm for 15 min before further analysis. To prepare the nuclear fraction, the cell pellet generated after initial centrifugation was washed with the hypotonic buffer three times and suspended in high-salt buffer (20 mM HEPES [pH 7.9], 1.5 mM MgCl₂, 0.2 mM EDTA [pH 8.0], 420 mM NaCl, 25% glycerol [vol/vol], 50 mM β -glycerophosphate, 1 mM NaF, and 1 mM Na₃VO₄), supplemented with dithiothreitol (1 mM), phenylmethylsulfonyl fluoride (1 mM), aprotinin (2 μ g/ml), leupeptin (2 μ g/ml), and soybean trypsin inhibitor (37.5 μ g/ml). The suspended cell pellet was incubated for 30 min on ice with occasional vortexing, and the nuclear fraction was collected after centrifugation at 13,000 rpm for 10 min. Protein content from each fraction was quantified by the Bradford assay (Bio-Rad) as specified by the manufacturer to ensure equal loading.

Immunoprecipitation

Vero E6 cells were transfected with pcDNA3.1(-)-M or pcDNA3.1(-). At 24 hr post-transfection, total cell lysate was prepared with immunoprecipitation assay (IPA) cell lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 2 mM EDTA, 1% sodium orthovanadate, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS) supplemented with phenylmethylsulfonyl fluoride (1 mM), aprotinin (2 μ g/ml), leupeptin (2 μ g/ml), and soybean trypsin inhibitor (37.5 μ g/ml). Monoclonal anti-IKK α or anti-IKK β (1–2 μ g) antibody (Cell Signal) was added to precleared cell lysate and incubated overnight at 4°C. Immune complexes were captured with 30 μ l of protein A-Sepharose (Santa Cruz) for 30 min at 4°C and washed five times with IPA buffer. Proteins were separated by SDS-PAGE, and then detected by Western blot analysis with anti-M polyclonal antibody.

Statistical Analysis

All experiments were repeated at least three times. Results are expressed as means \pm SEM. Statistical analysis was performed using the Statistical Package Social Sciences (SPSS) program version 11.5 by one-way analysis of variance (ANOVA) and significant differences among groups were determined by least significant difference (LSD). Differences were considered to be significant when $P < 0.05$.

RESULTS

SARS-CoV M Protein Suppresses NF- κ B Activity in Both HeLa and Vero E6 Cells

To investigate the effect of the M protein on NF- κ B activation, plasmids encoding both full-length and

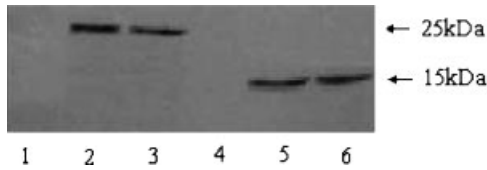


Fig. 1. Expression of M in transfected cells. HeLa or Vero cells were transfected with plasmids encoding M or M mutant containing amino acid residues 90–221. The cells were collected and subjected to Western blot analysis. **Lanes 1 and 4**, HeLa and Vero cells transfected with vector, respectively; **lanes 2 and 5**, HeLa cells transfected with M or M mutant respectively and **lanes 3 and 6**, Vero E6 cells transfected with M and M mutant, respectively.

C-terminal portion of M (M90) were transfected into HeLa and Vero cells along with a NF- κ B reporter plasmid. The expression of the SARS-CoV M protein and the mutant M90 in HeLa and Vero E6 cells were confirmed by Western blot (Fig. 1). In these two cell lines, luciferase

activities were lower when M was expressed, indicating that M protein expression results in a significant decrease in NF- κ B activity compared with the control (Fig. 2A). The inhibitory effect of M on NF- κ B activation is dose-dependent (Fig. 2B,C). To examine whether expression M can inhibit TNF- α induced NF- κ B activation in these two cell lines, the cells were treated with TNF- α after transfection. As shown in Figure 2D, expression of M blocked activation of luciferase expression under control of NF- κ B.

SARS-CoV M Protein Does Not Alter the Expression Levels of NF- κ B but Affects Their Nuclear Translocation

To understand the molecular basis of NF- κ B suppression by the SARS-CoV M protein, NF- κ B in Vero E6 cells were examined using Western blot with antibodies specific for the subunits of NF- κ B, p50, and p65. As

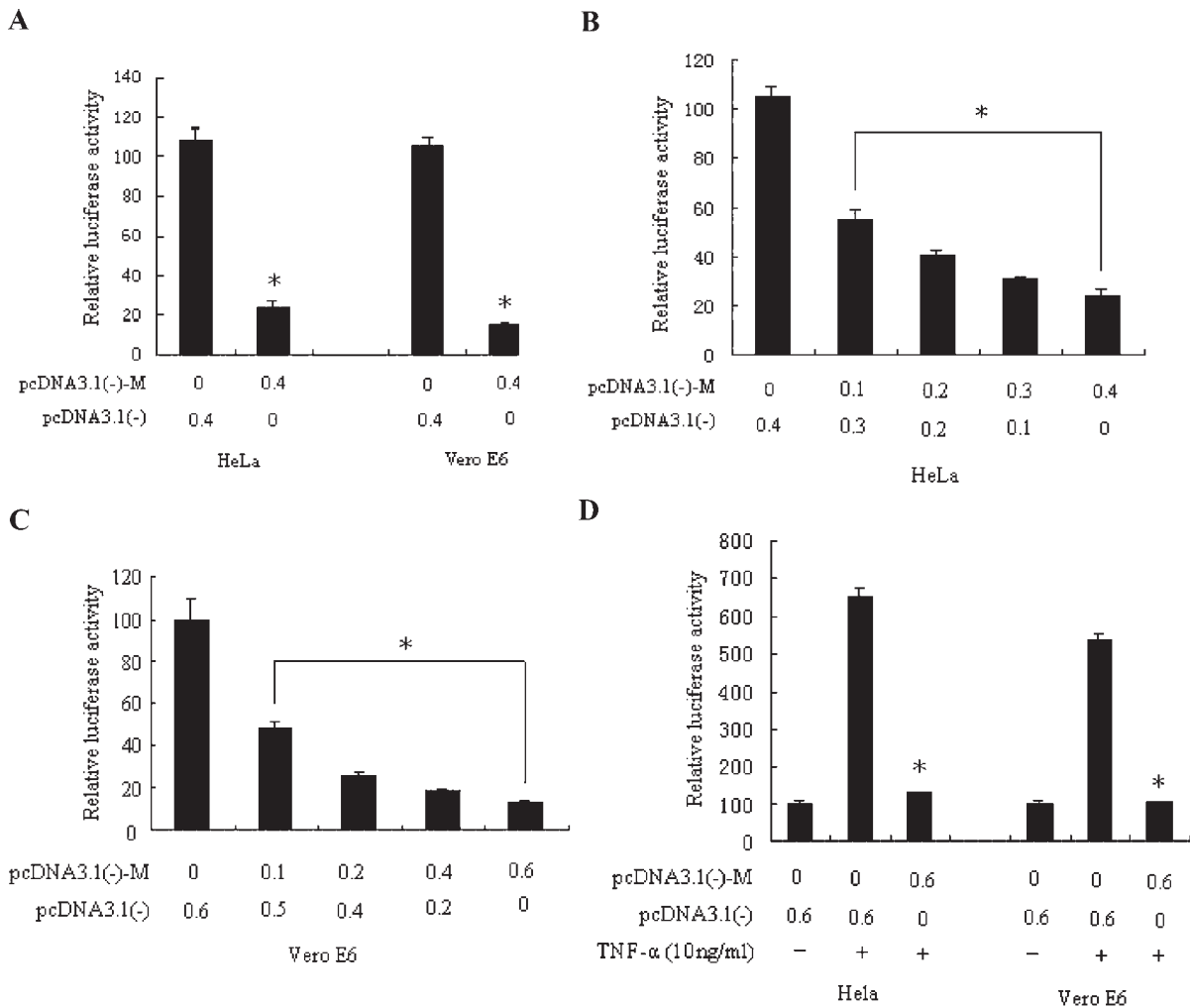


Fig. 2. Suppression of NF- κ B by the SARS-CoV M protein. **A:** HeLa and Vero E6 cells were transfected with 0.6 μ g of pNF- κ B-Luc and 0.6 μ g of pcDNA3.1(-)-M. Cells co-transfected with pNF- κ B-Luc and the pcDNA3.1(-) vector were used as negative control. Cells were harvested 24 hr after transfection and assayed for luciferase activity. HeLa (**B**) and Vero E6 (**C**) cells were transfected with different amounts of pcDNA3.1(-)-M along with the reporter plasmid pNF- κ B-Luc and relative luciferase activity was determined. **D:** HeLa and Vero E6 cells

were transfected with 0.6 μ g of pNF- κ B-Luc and 0.6 μ g of pcDNA3.1(-)-M. Cells transfected with pNF- κ B-Luc and the pcDNA3.1(-) vector were used as negative controls. Cells were treated with TNF- α (10 ng/ml) for 6 hr at 24 hr post-transfection and then assayed for luciferase activity. Luciferase activities correspond to an average of at least three independent experiments done in duplicate, and values are means \pm SEM. * $P < 0.01$ (significantly different from the control).

shown in Figure 3A, expression of the M protein did not affect expression levels of NF- κ B in Vero E6 cells. Activated of NF- κ B factors are translocated to nucleus. To determine the localization of NF- κ B, cytoplasmic and nuclear fractions were prepared and analyzed using Western blot. As shown in Figure 3B, expression of the M protein reduced translocation of p50 and p65 to the nucleus, and increased the amounts of cytoplasmic p50 and p65.

SARS-CoV M Protein Increases I κ B α Protein in Vero E6 Cells

To investigate how the M protein suppresses NF- κ B activity, expression of I κ B α was measured. Western blot analysis of cell lysates using I κ B α antibody showed that amount of I κ B α protein was increased in SARS-CoV M protein expressing Vero E6 cells in a dose-dependent manner (Fig. 4). So it is reasonable to predict that M protein can inhibit I κ B α protein phosphorylation and increase the amount of I κ B α protein.

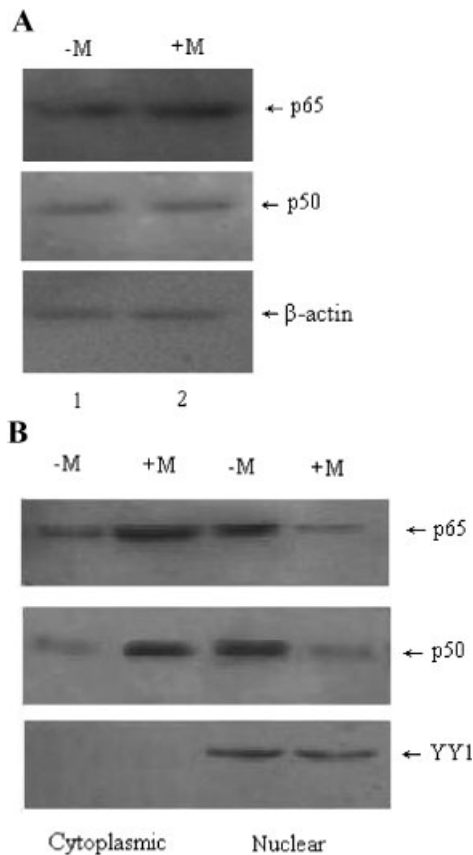


Fig. 3. The effects of the SARS-CoV M protein on NF- κ B in Vero E6 cells. **A:** Vero E6 cells were transfected with empty vector pcDNA3.1(-) as a control (**lane 1**) or with pcDNA3.1(-)-M (**lane 2**). Cell extracts were prepared and the expressed proteins were detected by Western blot with mouse monoclonal anti-p65, anti-p50, anti- β -actin antibody. **B:** Vero E6 cells were transfected with 0.8 μ g of either an empty vector or the pcDNA3.1(-)-M. At 48 hr post-transfection, cytoplasmic, and nuclear fractions were prepared as described in Section "Materials and Methods." Ten micrograms of cytoplasmic proteins and 50 μ g of nuclear proteins were analyzed by SDS-PAGE and Western blot with p65 or p50 antibody to reveal the location of NF- κ B. YY1, a nuclear protein, was used as a control.

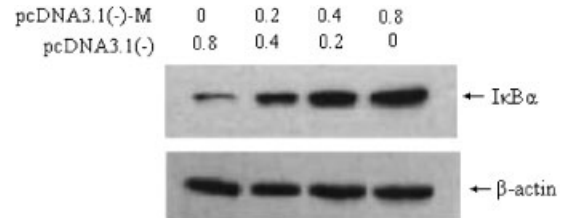


Fig. 4. The SARS-CoV M protein upregulated I κ B α protein level in Vero E6 cells. Vero E6 cells were transfected with different amounts of pcDNA3.1(-)-M. Cell extracts were prepared and the expressed proteins were determined using mouse anti-I κ B α antibody. β -Actin was used as a control.

SARS-CoV M Protein Physically Interacts With IKK β

To understand how the SARS-CoV M protein increases the amount of I κ B α protein, we examined the interaction between IKK signalsome and M protein by co-IP. SARS-CoV M protein expressing Vero E6 cells were lysed and the lysates were incubated with IKK α or IKK β antibody. Then the immune complex was analyzed by SDS-PAGE and Western blot with anti-M polyclonal antibody. As shown in Figure 5, SARS-CoV M protein co-precipitated with IKK β but not with IKK α , suggesting that SARS-CoV M protein can interact with IKK β .

SARS-CoV M Protein Suppresses Cox-2 Promoter and Decreases COX-2 Protein Expression

To investigate the roles of M protein in the expression of COX-2, the plasmid pcDNA3.1(-)-M was co-transfected into HeLa and Vero E6 cells with a reporter plasmid carrying the luciferase gene under the control of the Cox-2 promoter. Luciferase activity from M-transfected cells was about fivefold lower than that of

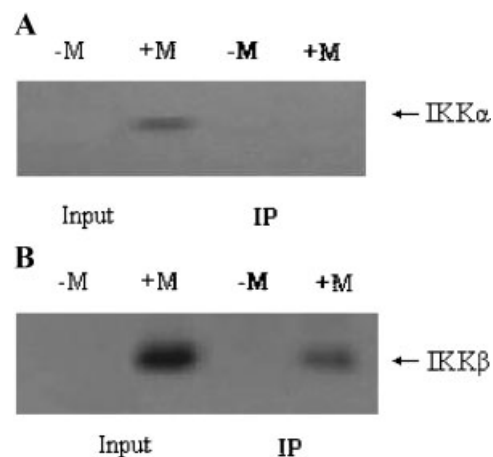


Fig. 5. The SARS-CoV M protein interacted with IKK β . Vero E6 cells were transfected with either 0.8 μ g of an empty vector or pcDNA3.1(-)-M. At 48 hr post-transfection, cell lysates were prepared for immunoprecipitation with monoclonal anti-IKK α (**A**) or anti-IKK β (**B**) antibodies. Immune complex captured by protein A-Sepharose was separated by SDS-PAGE and analyzed by Western blot. Blots were incubated with rabbit anti-M antibody. One-tenth of the total cell lysates used for immunoprecipitation was loaded as a positive control for IKK α and IKK β , respectively.

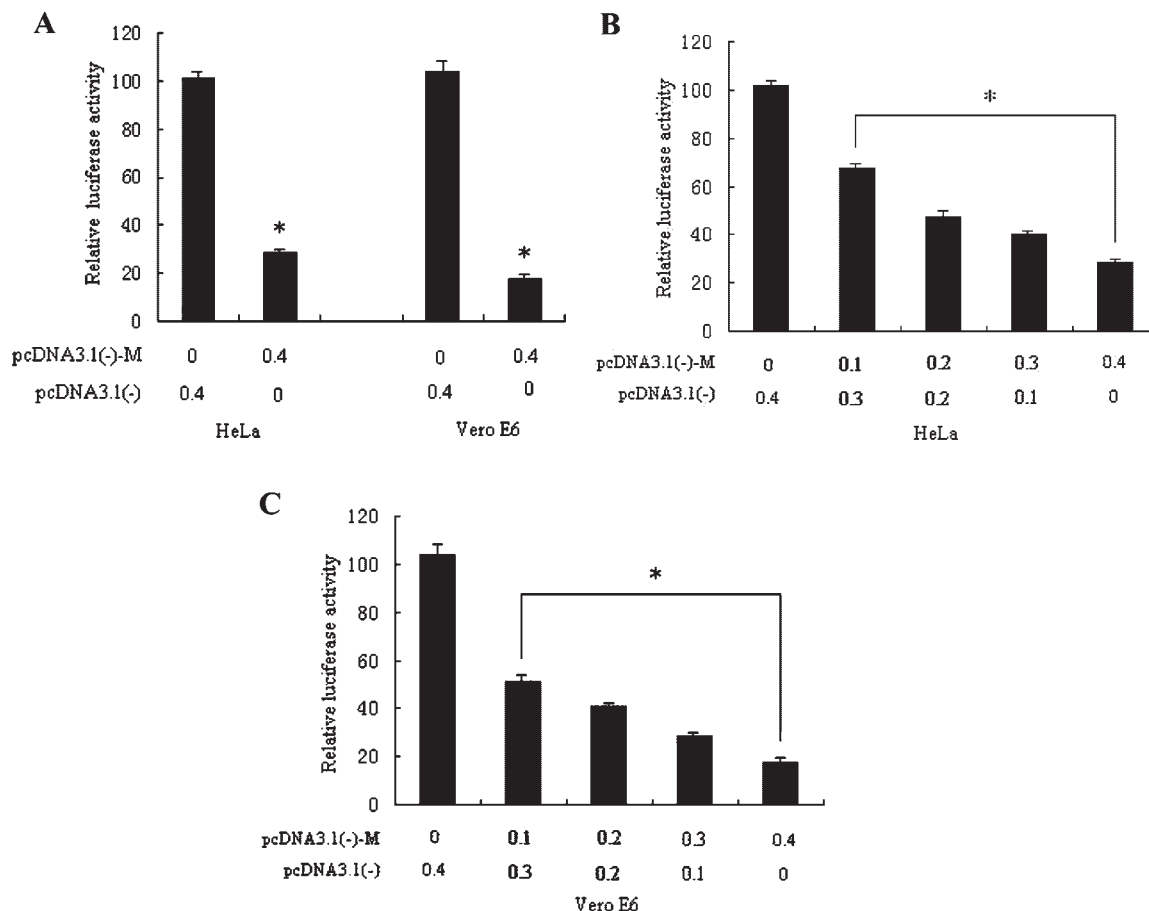


Fig. 6. Suppression of COX-2 expression by the SARS-CoV M protein. **A:** HeLa and Vero E6 cells were transfected with 0.6 μ g of pGL3 COX-2-Luc and 0.6 μ g of pcDNA3.1(-)M. Cells co-transfected with pGL3 COX-2-Luc and the pcDNA3.1 (-) vector were used as negative controls. Cells were harvested 24 hr after transfection and assayed for luciferase activity. HeLa (**B**) and Vero E6 (**C**) cells were

transfected with different amounts of pcDNA3.1 (-)M along with the reporter plasmid and relative luciferase activity was determined. Luciferase activities correspond to an average of at least three independent experiments done in duplicate, and values are means \pm SEM. * $P < 0.01$ (significantly different from the control).

vector control and suppression of Cox-2 promoter by the M protein was dose-dependent (Fig. 6). Furthermore, RT-PCR indicated that M protein caused downregulation of Cox-2 mRNA in a dose-dependent manner, suggesting that M protein expression downregulates COX-2 activity at the transcriptional level (Fig. 7). Western blot analysis of cell lysates using COX-2 antibody showed that COX-2 expression decreased in the M protein expressing cells. All these results demonstrated that the M protein of SARS-CoV is sufficient for the suppression of COX-2 promoter and protein expression.

The Carboxyl Terminal of SARS-CoV M Protein was Sufficient for the M Protein Function

Amino termini truncated M protein was expressed in both HeLa and Vero E6 cells, and luciferase activities showed that the truncated M protein suppressed NF- κ B activation and reduced the luciferase expression from the Cox-2 promoter-driven reporter gene to similar levels as that of wild type M protein (Fig. 8), indicating that the carboxyl terminal of SARS-CoV M

protein is sufficient for the M protein's inhibitory function.

DISCUSSION

In this study, we examined an inhibitory effect of SARS-CoV M protein on activation of NF- κ B, a key transcription factor in innate and adaptive immunity. Our data showed that the M protein suppressed NF- κ B activation and reduced expression of COX-2, an important inflammatory protein. We found that the M protein interacted with IKK β , which may in turn regulate NF- κ B activity. It is possible that the M protein sequesters IKK β and prevents formation of a fully functional IKK signalsome, which impairs NF- κ B signaling. Alternatively, it is possible that the M protein interferes with stoichiometric interactions between IKK β and other subunits of IKK signalsome.

NF- κ B activation plays an essential role in host immune responses to pathogens. The mice deficient in different members of the NF- κ B family are more susceptible to viral infection [Sha et al., 1995; Harling-McNabb et al., 1999; Tato and Hunter, 2002]. To evade

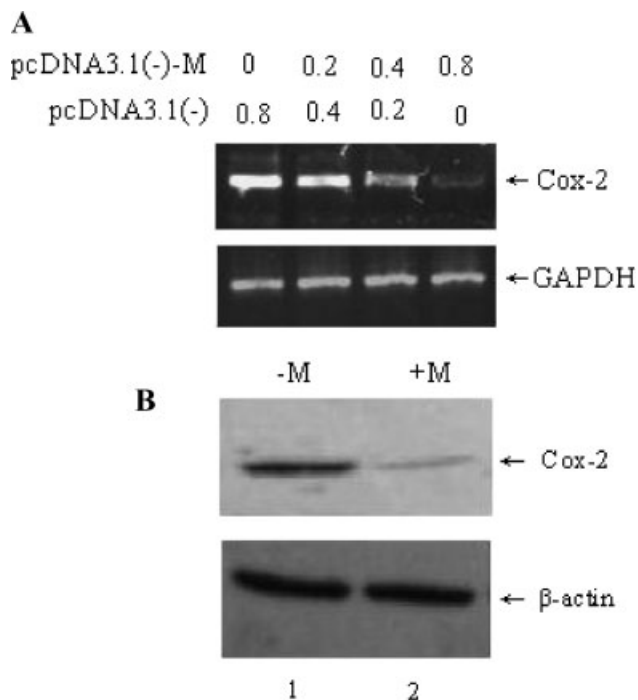


Fig. 7. The SARS-CoV M protein suppressed COX-2 protein expression in Vero E6 cells. **A:** Vero E6 cells were transfected with different amounts of pcDNA3.1(-)-M. RNA from the cells was purified and used for RT-PCR with Cox-2 specific primers. The RT-PCR products were resolved in agarose gel. GAPDH was used as a control. **B:** Western blot analysis of the expression of COX-2 protein. Vero E6 cells were transfected with empty vector pcDNA3.1(-) as a control (lane 1) or with plasmid pcDNA3.1(-)-M, expressing SARS-CoV M protein (lane 2). Cell extracts were prepared and the expression levels of Cox-2 proteins were determined using Western blot with rabbit anti-COX-2 antibody.

host immune responses, some viruses have evolved strategies to interfere with NF- κ B activation. For example, vaccinia virus encodes a viral homolog of the scaffolding protein MyD88, which is involved in IL-1-mediated activation of NF- κ B. The viral protein A52R acts as a dominant-negative form of MyD88, abrogating IL-1-mediated signaling, which is important for resistance to vaccinia virus [Bowie et al., 2000]. Cowpox, racoonpox, and some strains of vaccinia viruses were found to inhibit NF- κ B activation by interfering with I κ B α degradation [Oie and Pickup, 2001]. The African swine fever virus (ASFV) A238L protein, which is a homolog of I κ B and contains ankyrin repeats that bind to NF- κ B dimers, prevents NF- κ B nuclear translocation [Revilla et al., 1998]. HIV accessory protein Vpu has been shown to inhibit NF- κ B activation by interfering with b-TrCP-mediated degradation of I κ B α and to promote apoptosis of the infected cells by suppressing NF-dependent expression of anti-apoptotic factors [Akari et al., 2001; Bour et al., 2001]. In this study we demonstrated that the M protein can interact with IKK β and suppress the activation of NF- κ B.

NF- κ B along with other transcription factors regulates expression of Cox-2. Involvement of NF- κ B in COX-2 expression is well documented in a variety of cell types [D'Acquisto et al., 1997; Caivano et al., 2001]. Along with

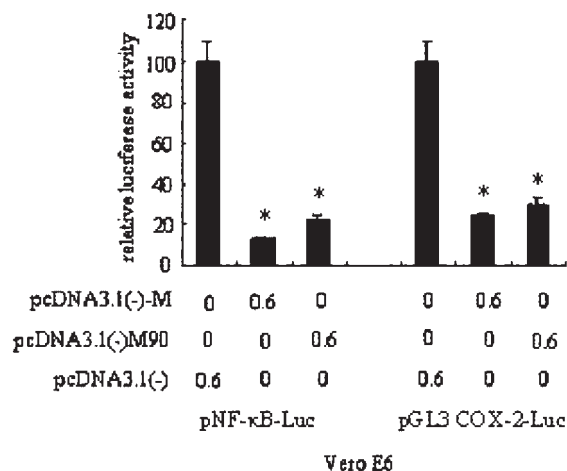


Fig. 8. The C-terminal of the SARS-CoV M protein was sufficient for the M protein mediated NF- κ B or COX-2 downregulation. Vero E6 cells were transfected with 0.2 μ g of reporter plasmids (pNF- κ B-Luc or pGL3 COX-2-Luc) and 0.6 μ g of pcDNA3.1(-)-M or pcDNA3.1(-)-M90. Cells transfected with the reporter plasmids and the pcDNA3.1(-) vector were used as negative controls. Cells were harvested 24 hr after transfection and assayed for luciferase activity. Luciferase activities correspond to an average of at least three independent experiments done in duplicate, and values are means \pm SEM. * P < 0.01 (significantly different from the control).

constitutively expressed COX-1, COX-2 metabolizes arachidonic acid to prostaglandin H₂, which is further metabolized to generate various prostanoids [FitzGerald, 2003]. Prostanoids are considered to promote inflammation by inducing vasodilation and increasing vascular permeability and cellular migration to the site of inflammation [Williams and Shacter, 1997; Dubois et al., 1998]. COX-2 protein plays a significant role in host immune response, in which its production is linked to exposure to catalase and peptidoglycan [Chen et al., 2004; Jang et al., 2004]. COX-2 has also specifically been shown to play a role in the pathogenesis of some viral infections [Steer and Corbett, 2003], notable examples being the dependence on COX-2 for replication of cytomegalovirus [Zhu et al., 2002] and the induction of COX-2 by primary human herpesvirus 6 infection [Janelle et al., 2002]. However, Epstein-Barr virus (EBV), a herpesvirus that establishes latency in white blood cells, has been shown to inhibit both COX-2 and prostaglandin E₂ in monocytes [Savard et al., 2000]. Similar to EBV, SARS-CoV M protein mediated Cox-2 downregulation may be beneficial to virus replication by limiting inflammatory responses.

SARS is characterized by a persistent fever and respiratory symptoms such as lung consolidation, lymphopenia, and respiratory failure in life-threatening cases [Peiris et al., 2003]. It has been postulated recently that the N protein of SARS-CoV activates the expression of Cox-2 by binding directly to regulatory elements for NF- κ B and CEBP [Yan et al., 2006]. Our studies have shown that SARS-CoV M protein suppresses NF- κ B activation and COX-2 expression. That the SARS-CoV M protein suppresses NF- κ B activation may be a strategy used by the virus to control the host cells to

facilitate the virus survival and replication. It was proposed that in early stage of viral infection, SARS-CoV evades protective immunity to their advantage. At a late stage of infection, SARS-CoV replicates at a high level and progeny viruses are released from host cells, SARS-CoV activates NF- κ B and upregulates COX-2 expression, then triggering antiviral activities of prostanoids. SARS sequelae, such as transendothelial migration of polymorphonuclear cells into the lung tissues, multiple organ dysfunction, and acute respiratory distress syndrome, have been postulated to be associated with cytokine and chemokine dysregulation.

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