Viral dsRNA activates mucin transcription in airway epithelial cells

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Abstract Double-stranded (ds) RNA is a biologically active component of many viruses including rhinoviruses infecting the upper respiratory tract. Mucus production is a common symptom of such infections. Here, we show that mucin, the glycoprotein subunit of mucus gels, is transcriptionally upregulated in an NF-κB- and p38-dependent manner when homogeneous cultures of epithelial cells are exposed to dsRNA. Furthermore, upstream of p38 in this system, dsRNA stimulates the extracellular release of ATP and activation of cell surface ATP receptors, which are G protein-coupled. This results in the stimulation of phospholipase C and protein kinase C. These findings suggest that ATP receptor antagonists could be used to modulate mucus production induced by virus.

Key words: (ds)RNA; Mucin; ATP; p38; NF-κB

1. Introduction

Over the course of evolution, mammalian hosts have acquired diverse mechanisms to limit viral infection. For example, binding of viral double-stranded (ds) RNA to protein kinase R (PKR) and oligoadenylate synthase stimulates these enzymes to block protein synthesis via effects on elongation initiation factor 2α [1] and RNase L [2]. The ultimate consequence of these effects is to induce apoptosis of the host cell [3], which limits spread of the virus.

Infection can also be limited by the production of antiviral cytokines. Among these are interferons, which impair viral gene expression [4,5], as well as interleukin (IL)-6 [6] and IL-12 [7], which contribute to the activation of cytotoxic T cells. Recent evidence demonstrates a role for Toll-like receptor 3 in the induction of interferon-β [8].

Here we report details of yet another mechanism used to limit infection, namely the production of a physical mucus barrier. Mucus overproduction has long been recognized as a symptom of respiratory viral infections, but its etiology is poorly understood. The present results are the first to show that mucin is induced as a direct effect of viral dsRNA on epithelial cells. As revealed below, the signaling pathways mediating mucin induction by dsRNA resemble those shown previously to mediate cytokine induction. These pathways include the activation of mitogen-activated protein kinases (MAPK) [9,10] and nuclear factor κB (NF-κB) [11–13].

In this report, we specifically show that the mucin gene *MUC2* is transcriptionally upregulated by dsRNA and that, just as in the case of IL-6, *MUC2* induction is an NF-κB-dependent and PKR-independent process that requires activation of the MAPK, p38 [3]. Furthermore, we show that signaling upstream of p38 requires the autocrine activation of ATP receptors. This mechanism had been previously implicated in the epithelial response to bacterial flagellin [14] and may also represent a general mechanism for transmitting dsRNA-initiated signals to MAPK and NF-κB.

2. Materials and methods

2.1. Reagents

dsRNA and single-stranded (ss) RNA were from Amersham Pharmacia Biotech and histone was from Gibco BRL. Chemical inhibitors caffeic acid phenethyl ester (CAPE), bisindolylmaleimide I, G06983, U73122, U73433, D609, suramin, and glibenclamide were from Calbiochem (La Jolla, CA, USA). 2-Aminopurine (2-AP), reactive blue 2 (RB2), pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), and gadolinium III chloride (GD3) were purchased from Sigma (St. Louis, MO, USA). SB202190 was purchased from A.G. Scientific (San Diego, CA, USA), and ATP was purchased from Invitrogen (Carlsbad, CA, USA).

2.2. Viral homologues

Both dsRNA and ssRNA were diluted to a concentration of 2 mg/ml in sterile H_2O and then stored at −20°C until diluted in serum-free medium (SFM) for use at 400 ng/ml. Histone was stored as 1 mg/ml and used at 1 μg histone/μg RNA.

2.3. Cell culture

Two epithelial cell lines were used in these studies. HM3 cells (from colon) were maintained in Dulbecco's modified Eagle's medium. NCIH292 cells (from airway) were maintained in RPMI 1640 medium. All media contained 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin.

2.4. Cloning and sequencing of the 5′-flanking region of MUC2, plasmid construction, transfection, and luciferase assay

The 5′-flanking region of the human mucin gene *MUC2* was cloned

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Abbreviations: dsRNA, double-stranded RNA; NF-κB, nuclear factor κB; PKR, RNA-activated protein kinase; PLC, phospholipase C; PKC, protein kinase C; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; IL-6, interleukin-6; ssRNA, single-stranded RNA; CAPE, caffeic acid phenethyl ester; 2-AP, 2-aminopurine; RB2, reactive blue 2; GD3, gadolinium III chloride; RT-PCR, reverse transcription polymerase chain reaction; RPA, RNase protection assay; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling; SFM, serum-free medium; SAPK, stress-activated protein kinase; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; GPCR, G protein-coupled receptor; LPS, lipopolysaccharide; SFM, serum free medium

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and stably transfected into HM3 cells and used as described [14]. Stable transfectants are called HM3-MUC2 cells. Histone plus either dsRNA or ssRNA in SFM was incubated with the cells for specified times and the cells were then harvested for luciferase assay. Chemical inhibitors were preincubated with cells for 30–60 min.

2.5. Reverse transcription polymerase chain reaction (RT-PCR)

RNA was isolated from cells using Trizol (Gibco BRL) and 5 μg was used for reverse transcription using Superscript reverse transcriptase (Gibco BRL). One tenth of the reaction product was used as a template for PCR amplification with Taq polymerase (AmpliTaq, Roche). Custom primers for *MUC2* gene were: forward 5′-CAGAGGGCAGAACCCGATACC-3′, reverse 5′-ACATCGGGCTGGTTGATCTTC-3′. GAPDH primers and 18S primers were used as controls.

2.6. RNase protection assay (RPA)

The *MUC2* probe for RPA was constructed using PCR with primers corresponding to nucleotides 423–639 of the cDNA sequence as previously described [15]. The resulting 216 bp cDNA was TA-cloned into pCRII vector (Invitrogen). The vector was linearized with *Bam*HI in a 100 μl volume at 37°C overnight. The DNA was treated with proteinase K before extracting with equal volumes of phenol/chloroform/isoamyl alcohol. The extract was precipitated with ammonium acetate and ethanol for 15 min at −80°C and redissolved in diethylpyrocarbonate-treated water. Riboprobe was synthesized in the presence of ^{32}P-labelled UTP using linearized and purified DNA template. It was then subjected to RNase-free DNase I at 37°C, phenol extracted and ethanol precipitated. The pellet was resuspended in gel loading buffer. The probe was purified on a polyacrylamide gel run at 300 V for 1 h. The target band on the gel was visualized by exposure to X-omat AR film (Kodak). The band was excised and incubated in elution buffer (RPA II kit, Ambion). The purified probe was diluted in 1×hybridization buffer so that each sample had 500 000 cpm. After 10 μl of probe was added, the samples were heated at 95°C for 5 min before hybridizing at 45°C overnight. After hybridization, 200 μl of diluted RNase A and T (RPAII kit, Ambion) were added to each sample and they were incubated at 37°C for 30 min. The samples were precipitated and redissolved in 4 μl of gel loading buffer, denatured at 95°C for 5 min before running on a 0.5 mm thick 6% polyacrylamide/8 M urea gel at 1500 V for 2 h. The gel was dried and exposed to X-omat AR film at −80°C for 2–7 days.

2.7. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) assay

NCIH292 cells were grown to 50–60% confluence in four chamber slides (LabTek II, Nalge Nunc International) and were pretreated with or without 2-AP for 1 h prior to addition of dsRNA (400 ng/ml). After 24 h of incubation with dsRNA, an apoptosis assay kit (ApopTag, Intergen) using indirect fluorescent staining of cells was used to detect fragmentation of nuclei undergoing apoptosis. The fixed slides were then viewed under a fluorescence microscope (Nikon Eclipse E600). The image was captured using a Zeiss Axiocam digital camera and software.

2.8. Immunoblots and kinase assays

Protein was isolated from cells and probed with specific antibodies for detection of phosphorylated residues. Cell lysates were pre-cleared by centrifugation at 10 000 rpm for 10 min at 4°C and total protein concentrations were determined using the Bradford protein assay (Bio-Rad). Equal amounts of lysate were run directly with the indicated antibodies. Lysates were incubated with anti-p38 MAPK antibody (New England Biolabs) and protein A agarose beads (Santa Cruz Biotechnology) overnight at 4°C. The lysate–antibody–bead complex was spun down and washed three times with lysis buffer (20 mM Tris–HCl, 150 mM NaCl, 0.5% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 1 mM EDTA, 1 mM sodium orthovanadate). Following the final wash, 40 μl of SDS gel loading buffer was added, the mixture was heated at 100°C for 3 min, and proteins were resolved by SDS–polyacrylamide gel electrophoresis (PAGE). For immunoblot analysis, proteins were transferred to nitrocellulose membranes using the Bio-Rad Mini Trans-Blot Electrophoretic Transfer Cell. Membranes were blocked in phosphate-buffered saline containing 0.1% Tween (PBS/Tween) and supplemented with 5% bovine serum albumin. After 1 h at room temperature, blots were washed with PBS/Tween and incubated with the appropriate antibody overnight at 4°C. After removing primary antibody with several washes of PBS/Tween, the blot was placed in the appropriate horseradish peroxidase-conjugated secondary antibody for 45 min. After several washes, the antibody–antigen complexes were visualized using the ECL chemiluminescence detection system (Amersham Life Sciences). For detection of Jun N-terminal kinase (JNK) activity, a stress-activated protein kinase (SAPK)/JNK assay kit (Cell Signaling Technology) was used to 'pull down' SAPK using c-Jun fusion protein beads prior to performing a kinase reaction in the presence of ATP. After running the reaction products on SDS–PAGE, c-Jun phosphorylation was detected using a phospho-c-Jun antibody.

2.9. ATP assay

Direct measurement of ATP release was performed by growing NCIH292 cells to confluence in 12 well culture plates at 37°C, and then placing them in SFM overnight. The next day, cells were treated with ssRNA or dsRNA in a total of 1 ml medium per well. At indicated times after RNA addition, 100 μl volumes were carefully removed from the surface without perturbing the cells and added to 100 μl luciferin/luciferase reagent (ENLITEN ATP assay system, Promega) before reading in a Monolight luminometer.

2.10. Statistics and densitometry

All data are expressed as mean ± S.E.M. One way analysis of variance was used to determine statistically significant differences between groups. A probability of <0.05 for the null hypothesis was accepted as indicating a statistically significant difference. Densitometry measurements of immunoblot bands were carried out using the histogram calculator in Adobe Photoshop (Adobe Systems). Blots

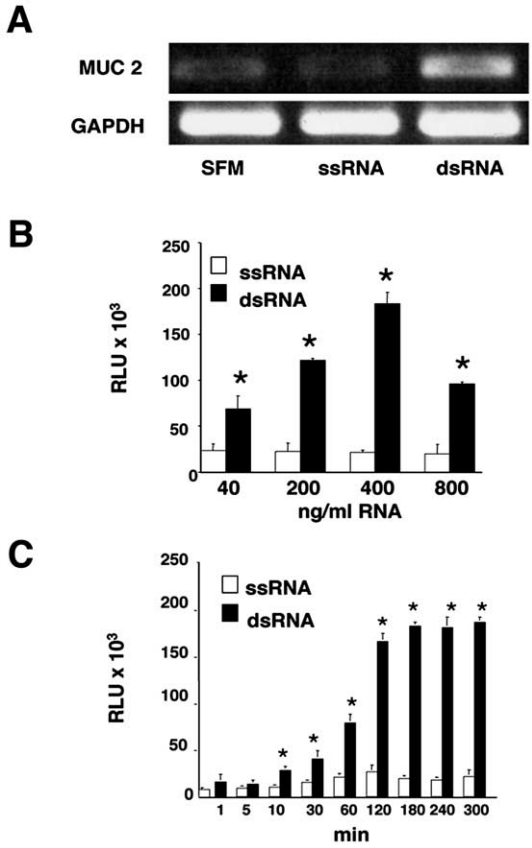

Fig. 1. dsRNA stimulates mucin gene (*MUC2*) transcription. A: RT-PCR shows induction of endogenous *MUC2* by dsRNA (4 h incubation), HM3 cells. B: *MUC2* promoter is stimulated by dsRNA (4 h incubation) in a dose-dependent manner, as reflected by activity of the reporter gene, luciferase, in HM3-MUC2 cells. C: Time-dependent activity of *MUC2* promoter in HM3-MUC2 cells exposed to dsRNA (400 ng/ml). Data points are averages from three separate experiments, each containing $n=3$ samples. An asterisk signifies dsRNA and ssRNA responses are different ($P<0.05$).

were normalized after reprobing with appropriate antibody to ensure equal loading.

3. Results

3.1. dsRNA stimulates mucin synthesis in epithelial cells

Mucin production increases in the respiratory tract during virus infection [16] but whether this is due to direct effects of virus or is secondary to inflammation is unknown. Here, we tested the direct effects of viral dsRNA on mucin synthesis in two human epithelial cell lines (HM3 and NCIH292). Because preliminary studies showed that responses to rhinovirus dsRNA [17] were the same as those to synthetic dsRNA, we used the synthetic form for convenience. Using ssRNA as a negative control, we observed increased steady-state levels of *MUC2* RNA in response to dsRNA (Figs. 1A and 2B). These results support the view that mucin overproduction during viral infection is at least partly due to a direct effect of dsRNA on epithelial cells. We illustrate data from HM3 and NCIH292 cells interchangeably below, as the two cell lines showed the same pattern of response to inhibitors.

3.2. Mucin upregulation by dsRNA is controlled at the level of transcription

To determine whether mucin induction by virus, like that by bacteria [14,18], is controlled at the level of transcription, we performed experiments using HM3 cells stably transfected with a luciferase reporter under the control of a 2.8 kb fragment of the *MUC2* 5′-flanking region [14,18]. In response to dsRNA, luciferase activity increased in both a dose- and time-dependent manner (Fig. 1B,C), strongly suggesting that the steady-state responses shown in Figs. 1A and 2B are mediated by transcriptional induction involving response elements present in the 5′-flank of *MUC2*.

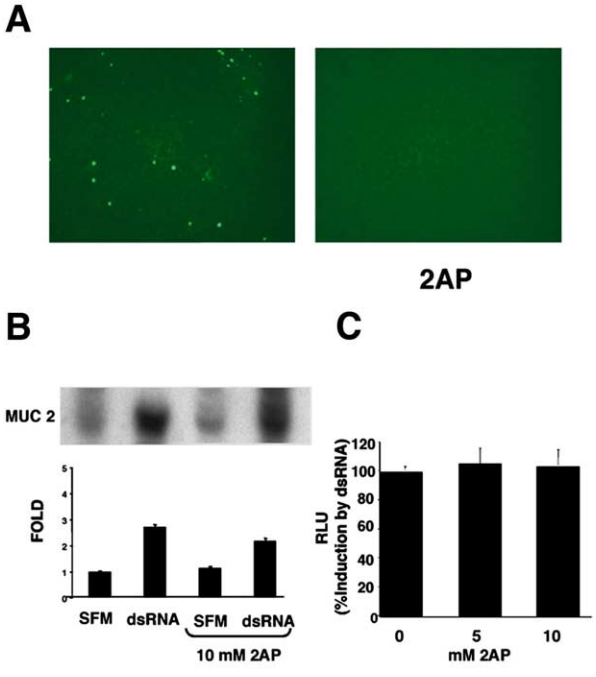

Fig. 2. PKR inhibitor 2-AP inhibits apoptosis but not *MUC2* induction by dsRNA. A: TUNEL assay shows apoptotic cells following 24 h incubation with dsRNA (left panel), but not when dsRNA exposure is preceded (1 h) and coextensive with exposure to 2-AP (5 mM; right panel), HM3 cells. B: RPA shows *MUC2* RNA induction by dsRNA (4 h incubation). 2-AP treatment (as in A) does not inhibit the response. Densitometry data in lower panel are controlled for sample loading using a probe for the housekeeping gene, cyclophilin, HM3 cells. C: *MUC2* promoter activity monitored by luciferase is unaffected by 2-AP in HM3-MUC2 cells (administered as in A).

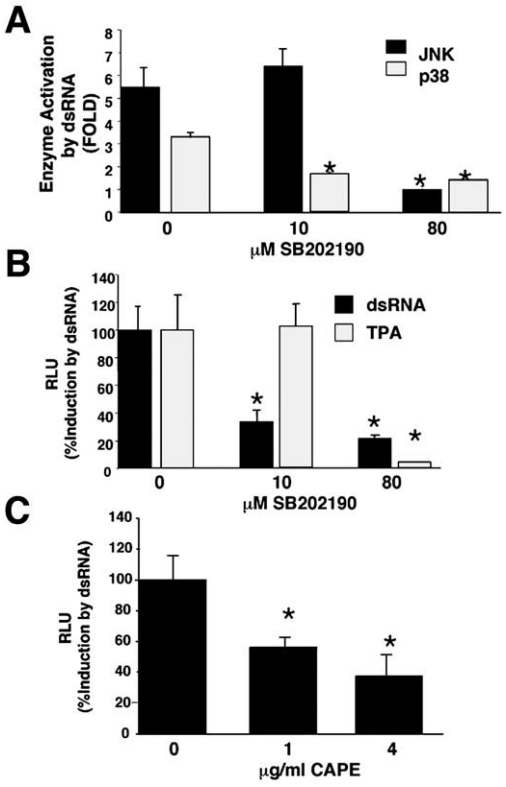

Fig. 3. p38 and NF-κB are required for mucin induction by dsRNA. A: Densitometry values reflecting p38 phosphorylation and JNK activity in response to dsRNA, in the presence and absence of kinase inhibitor SB202190 (1 h preincubation and 1 h co-exposure with dsRNA) in NCIH292 cells. In agreement with [21], p38 is inhibited at low, and JNK at high, concentrations of this drug. B: Effect of low and high doses of inhibitor on mucin induction by dsRNA and TPA. Data are averages of three separate experiments and were normalized in A by reprobing immunoblots with anti-p38 to control for sample loading. C: *MUC2* promoter activity monitored by luciferase is inhibited by the NF-κB inhibitor CAPE (1 or 4 μg/ml; 1 h pretreatment and 4 h co-treatment with dsRNA) in HM3-MUC2 cells. An asterisk signifies effects of inhibitors were significant at the level of $P < 0.05$.

3.3. Mucin induction by dsRNA is PKR-independent

Antiviral responses, once thought to be principally dependent on the dsRNA-dependent kinase, PKR, have been shown in some cases to be PKR-independent and to involve other upstream mediators [9,10]. The compound 2-AP inhibits dsRNA-initiated, PKR-dependent processes such as translational inhibition [19] and apoptosis [20]. In our model, despite 2-AP's ability to block dsRNA-induced apoptosis (Fig. 2A), it had no effect on mucin induction (Fig. 2B,C). Thus, the induction of mucin by dsRNA does not require PKR.

3.4. Mucin induction by dsRNA is p38- and NF-κB-dependent

In agreement with data previously obtained in fibroblasts [3,10], we found that dsRNA stimulates JNK and p38 in epithelial cells (Fig. 3A). In addition, consistent with [21], we

found that the inhibitor SB202190 selectively inhibits p38 at low doses, while it inhibits both p38 and JNK at 80 μM (Fig. 3A). Because mucin induction by dsRNA, but not 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (which acts through JNK), was inhibited at the lower dose (Fig. 3B), we conclude that mucin induction by dsRNA requires activation of p38. As suggested [9], the same upstream dsRNA-sensing mechanisms that stimulate p38 may also stimulate NF-κB. Indeed, data in Fig. 3C illustrate that mucin induction, already shown to be sensitive to the p38 inhibitor SB202190, is also sensitive to CAPE, which inhibits the binding of NF-κB to DNA. A requirement for NF-κB is consistent with the presence of an NF-κB site at approximately (−) 1.5 kb in the *MUC2* sequence driving the luciferase reporter [22]. Taken together, and in agreement with [23], these data suggest the existence of a linear pathway in which p38 acts upstream of NF-κB to stimulate gene expression.

3.5. *dsRNA activates mucin via protein kinase C (PKC), phospholipase C (PLC) and a G protein-coupled receptor (GPCR)*

To define additional signaling components, we screened a broad range of compounds for their ability to block mucin induction by dsRNA. Among the most potent were the PKC inhibitors bisindolylmaleimide I and GO6983 (Fig. 4A). Sensitivity to these drugs identifies PKC, for the first time, as a contributor to antiviral signaling. That PKC's specific role in dsRNA signaling may be to stimulate p38 is possible based on results in cardiac myocytes [24].

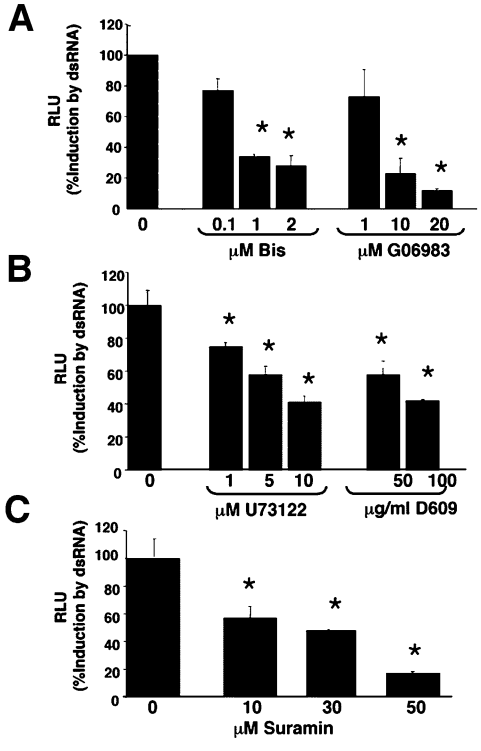

Fig. 4. PKC, PLC, and GPCR inhibitors inhibit *MUC2* induction by dsRNA. A: Effects of PKC inhibitors bisindolylmaleimide (Bis) and GO6983. B: Effects of PLC inhibitors U73122 and D609. C: Effect of the GPCR inhibitor suramin. All administered as 1 h pretreatment and 4 h co-treatment with dsRNA in HM3-MUC2 cells. An asterisk signifies effects of drugs were significant at the level of $P < 0.05$.

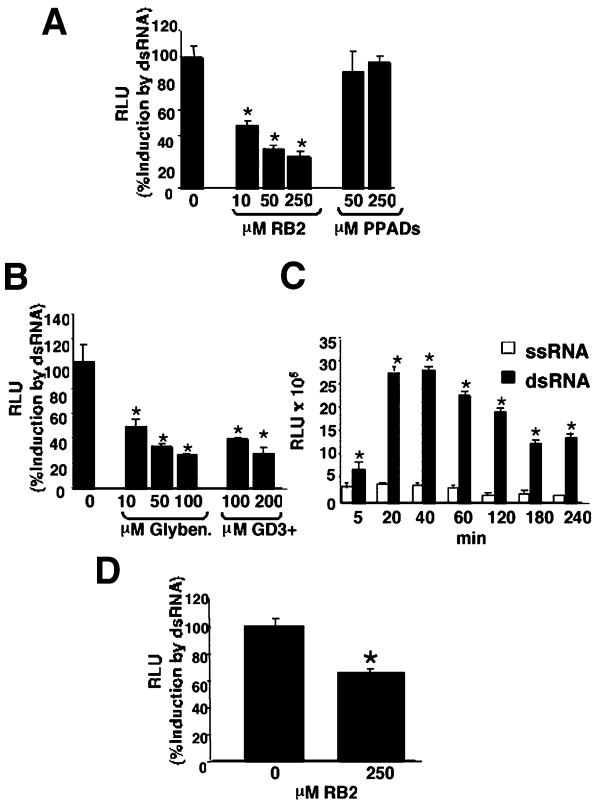

Fig. 5. Evidence for involvement of nucleotide receptors in the response to dsRNA. A: *MUC2* promoter activity monitored by luciferase is inhibited by RB2, an inhibitor of P2Y nucleotide receptors, but not PPADS, an inhibitor of P2X nucleotide receptors (4 h incubation with dsRNA). B: Same as in A, showing inhibition of *MUC2* promoter activity by ATP conductance-blocking drugs, glibenclamide and GD3+. C: Time-dependent luciferin/luciferase assay comparing ATP release from cells stimulated by ssRNA and dsRNA. D: Luciferase activity driven by NF-κB response elements stimulated by dsRNA (4 h incubation) with and without 1 h pretreatment with RB2. Mucin and NF-κB induction are shown in the presence and absence of inhibitors administered as 1 h pretreatment and 4 h co-treatment with stimulus (dsRNA) in HM3-MUC2 cells. An asterisk signifies effects of drugs were significant at the level of $P < 0.05$.

We next addressed the question of how dsRNA activates PKC. This enzyme is commonly activated by diacylglycerol, a product of phosphatidylinositol 4,5-bisphosphate cleavage by PLC. Supporting the involvement of such a mechanism in dsRNA signaling, the PLC inhibitors U73122 and D609 strongly attenuated mucin induction (Fig. 4B). Furthermore, attenuation of the response in the presence of the GPCR-blocking agent suramin (Fig. 4C) suggested that dsRNA-initiated signaling includes a GPCR–PLC–PKC signaling cassette.

3.6. *Nucleotide receptor signaling is required for the induction of mucin by dsRNA*

Some of the most extensively analyzed GPCRs on the surface of respiratory epithelial cells are members of the P2Y family of nucleotide receptors responsible for chloride and macromolecule secretion [25]. We recently reported that such receptors mediate epithelial cell responses to bacterial flagellin [14]. A role for P2Y receptors was equally apparent in this study. Thus, P2Y, but not P2X, receptor antagonists

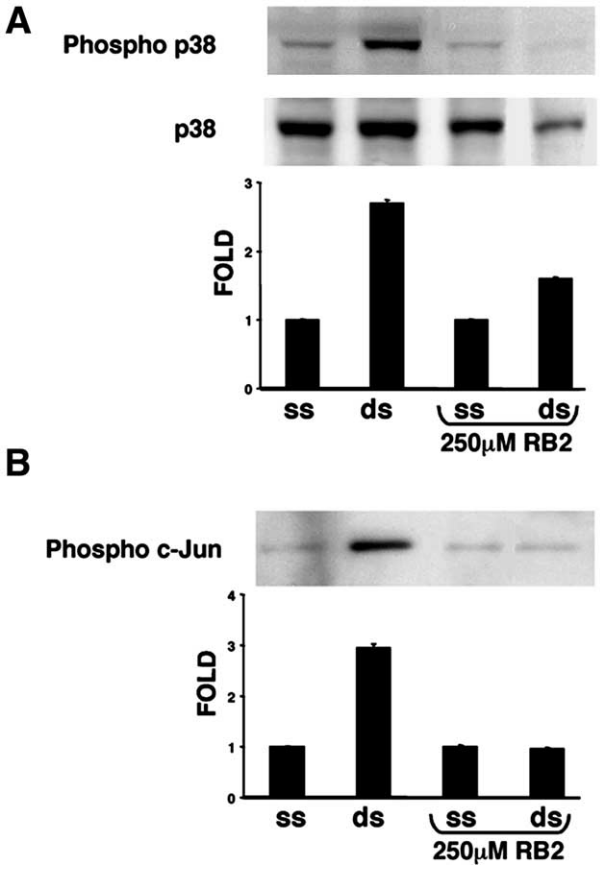

Fig. 6. Evidence for involvement of nucleotide receptors in the activation of p38 and JNK by dsRNA. A: As shown previously [3], p38 is phosphorylated by dsRNA. Here p38 phosphorylation is shown in NCIH292 cells; the response is blocked by nucleotide receptor antagonist RB2. Band density in anti-phospho-p38 immunoblot (upper panel) is normalized with respect to band density in anti-p38 immunoblot (lower panel) prior to quantification (histogram). B: As in A, but data here are from a JNK kinase assay in which JNK was pulled down from equal amounts of protein extract using c-Jun fusion protein beads and the c-Jun fusion protein was used as a substrate for kinase activity. c-Jun phosphorylation was monitored at Ser63 by Western blotting and chemiluminescence. All data are typical of at least two experiments, each with $n = 2$. Kinase activities were measured in the presence and absence of RB2 administered as 1 h pretreatment and 1–2 h co-treatment with dsRNA.

(RB2 vs. PPADS) strongly inhibited mucin induction by dsRNA (Fig. 5A). The involvement of P2Y receptors implies that extracellular nucleotide is somehow presented to these receptors during dsRNA exposure. Hypothesizing that dsRNA might cause ATP to be released extracellularly, we examined the effect on mucin induction of glibenclamide and GD3+, two compounds known to inhibit ATP transport across cell membranes [26,27]. The inhibitory effect of both compounds (Fig. 5B) supported a role for ATP in the response, and implied that ATP is, indeed, released from cells upon dsRNA exposure. Direct evidence for this was provided by an assay of ATP in the medium of dsRNA-exposed cells (Fig. 5C). Importantly, mucin induction by irrelevant stimuli (e.g. lipopolysaccharide (LPS)) was unaffected by RB2, glibenclamide, and gadolinium (not shown), indicating specificity.

3.7. Nucleotide receptors are also required for NF-κB activation in response to dsRNA

NF-κB is required for the induction of both mucin (Fig. 3C) and cytokines [3,10] and may coordinate other dsRNA-evoked responses as well. That NF-κB activity was, itself, reduced by RB2 (Fig. 5D) suggested that nucleotide receptors might control a wide array of dsRNA- and NF-κB-dependent responses.

3.8. dsRNA also stimulates p38 and JNK via nucleotide receptors

JNK has been implicated in the induction of interferon [10], and p38 has been implicated in the induction of IL-6 [3], by dsRNA. Further supporting a general role for nucleotide receptors in dsRNA signaling are data showing that the activation of these two kinases was strikingly reduced in the presence of RB2 (Fig. 6A,B).

4. Discussion

Apoptosis and cytokine production are well-documented responses of host cells to viral dsRNA. The present study identifies mucin induction as a third major defensive response. Our results show that dsRNA stimulates mucin transcription in an NF-κB-dependent, p38-dependent, PKR-independent manner similar to that controlling the induction of IL-6 [3]. The present work also provides new information regarding mechanisms of signal transduction upstream of p38 in this system. Specifically, our data indicate that dsRNA causes ATP to be released extracellularly and that this is followed by activation of cell surface ATP receptors, which are G protein-coupled. Like other examples of GPCR activation, this results in the stimulation of PLC and PKC. Downstream events relevant to mucin induction include activation of p38 and NF-κB. Our model is shown in Fig. 7.

A conclusion of the present study is that p38, but not JNK, is required for mucin induction. This is based on use of the compound SB202190. Earlier work had shown that SB202190

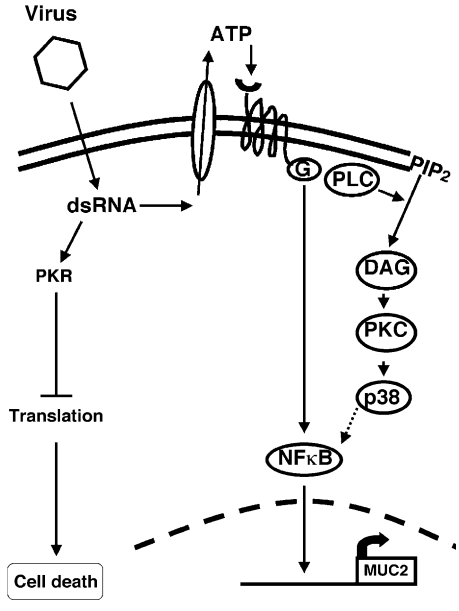

Fig. 7. Model of host inflammatory response to viral dsRNA in epithelial cells. See explanation in the text.

inhibits both p38 and JNK at ~80 μM, but inhibits p38 exclusively at ~10 μM [21]. Data in Fig. 3 confirm this. Moreover, the data clearly show that mucin induction by dsRNA is inhibited at the lower dose, implicating p38 in the response.

As mentioned above, it had been shown previously that p38 was required for IL-6 induction by dsRNA [3]. Although that study also demonstrated activation of two kinases acting immediately upstream of p38 (MKK3 and MKK6), it did not address mechanisms acting further upstream. Our evidence that mucin induction is blocked by bisindolylmaleimide and GO6983 implicates PKC in this pathway. Based on previous evidence that PKC is capable of activating p38 [28], we postulate that the role of PKC in this pathway is to stimulate p38. Furthermore, based on evidence that p38 is required for activation of NF-κB in the context of *Haemophilus* infection [23], we speculate that this interaction may also occur in pathways stimulated by dsRNA.

A common mechanism for activating PKC is via PLC. That this, too, plays a role in dsRNA-induced responses was indicated by results obtained using compounds U73122 and D609 (Fig. 4). PLC involvement had been observed previously in a model of mucin induction by bacterial flagellin [14]. In that model, PLC activation was a consequence of autocrine/paracrine stimulation of P2Y nucleotide receptors. The present results show that this also occurs in the context of dsRNA: thus, mucin induction by dsRNA was blocked by the P2Y receptor antagonist RB2 as well as the ATP conductance inhibitors gadolinium and glibenclamide (Fig. 5). Importantly, these drugs did not block mucin induction by LPS, illustrating the diversity of mechanisms by which mucin can be induced by pathogens at the cell surface.

The notion that autocrine/paracrine nucleotide signaling mediates host defense responses is novel in the context of epithelial cells, but had been described earlier for mast cells, macrophages, eosinophils and lymphocytes [29–32]. Paracrine signaling, in particular, may be of great benefit in propagating defensive responses from one or a few pathogen-exposed cells to neighboring cells in a mucosal epithelium. Although the means by which ATP is released from mucosal cells is unclear, it has been suggested that ATP extrusion is mediated by the ABC transporter family, at least one member of which, the cystic fibrosis transmembrane conductance regulator, is present in mucosal tissues [33,34].

In summary, our data provide details of a novel signaling pathway responsible for the induction of mucin in epithelial cells by viral dsRNA. This pathway may underlie mucus overproduction during respiratory viral infections, in which case P2Y nucleotide receptors, PLC, PKC and p38 could be drug targets for relief of the common cold. Moreover, because the activation of p38 and JNK is dependent on nucleotide receptor signaling, P2Y antagonists might play a relatively broad role in controlling viral pathogenesis.

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