

Corticosteroids inhibit rhinovirus-induced intercellular adhesion molecule-1 up-regulation and promoter activation on respiratory epithelial cells

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Background: Rhinoviruses are associated with the majority of asthma exacerbations. To date, the pathogenesis of virus-induced asthma exacerbations is still unclear, and no safe effective therapy is available. Intercellular adhesion molecule-1 (ICAM-1) has a central role in inflammatory cell recruitment to the airways in asthma and is the receptor for 90% of rhinoviruses. We have previously shown that rhinovirus infection of lower airway epithelium induces ICAM-1 expression by a transcriptional mechanism that is critically nuclear factor- κ B-dependent.

Objective: The purpose of this study was to investigate the effect of systemic (hydrocortisone [HC], dexamethasone [DM]) and topical (mometasone furoate [MF]) corticosteroids on rhinovirus-induced ICAM-1 up-regulation.

Methods: Cultured primary bronchial or transformed (A549) respiratory epithelial cells were pretreated with corticosteroids for 16 hours and infected with rhinovirus type 16 for 8 hours. ICAM-1 surface expression was evaluated by flow cytometry. In A549 cells ICAM-1 messenger RNA was evaluated by specific reverse transcription-PCR and promoter activation by chloramphenicol acetyltransferase assay.

Results: We observed inhibition of rhinovirus-induced ICAM-1 up-regulation with corticosteroid pretreatment in both primary bronchial epithelial and A549 cells. In A549 cells systemic and topical corticosteroids demonstrated a dose-dependent inhibition with similar efficacy (inhibitory concentration 50% 10^{-10} mol/L, 10^{-11} mol/L, and 10^{-11} mol/L for HC, DM, and MF respectively). MF also inhibited ICAM-1 messenger RNA induction by rhinovirus infection in a dose-dependent manner. MF completely inhibited rhinovirus-induced ICAM-1 promoter activation. HC, DM, and MF had no direct effect on rhinovirus infectivity and replication in cultured cells.

Conclusion: Corticosteroids decrease rhinovirus-induced ICAM-1 up-regulation in respiratory epithelial cells and modulate pretranscriptional mechanisms. This effect may be important for the therapeutic control of virus-induced asthma exacerbations. (*J Allergy Clin Immunol* 2000;105:318-26.)

Key words: Asthma, rhinovirus, intercellular adhesion molecule-1, corticosteroids

Adhesion molecule-mediated cell-cell and cell-matrix interactions are known to play critical roles in inflammatory cell recruitment and activation.¹ Among the adhesion molecules, intercellular adhesion molecule-1 (ICAM-1), a surface glycoprotein that belongs to the Ig supergene family, is a ligand for the integrin receptors leukocyte function-associated antigen-1 and Mac-1, which are expressed on leukocytes.^{2,3} ICAM-1 is expressed on several cell types, including the respiratory epithelium where its expression can be up-regulated by a number of proinflammatory cytokines.⁴ In the human respiratory tract type II epithelial cells have recently been demonstrated to express constitutive surface ICAM-1^{5,6} and airway epithelial cells express detectable ICAM-1 in primary cultures.^{7,8} We have recently found that in bronchial and pulmonary epithelial cells ICAM-1 surface expression is increased by rhinovirus infection through a transcriptional mechanism critically dependent on nuclear factor- κ B (NF- κ B) activation.⁸ This finding is of considerable interest because ICAM-1 is also the cellular receptor that 90% of rhinovirus serotypes (major group) use to enter and infect epithelial cells.⁹ Rhinoviruses are the etiologic agents of the most common acute infectious disease in humans, the common cold,¹⁰ and are also responsible for the majority of asthma exacerbations in children and in adults, both in the community^{11,12} and in more severe episodes requiring hospitalization.¹³

The precise mechanisms by which rhinovirus infection can promote asthma exacerbations are largely unknown. Inflammatory changes that follow rhinovirus infection are thought to play a driving role,^{14,15} with airway epithelial cells triggering local inflammation through the production of inflammatory cytokines or mediators. Indeed, studies with primary or transformed respiratory epithelial cells indicate that in vitro infection with rhinovirus induced secretion of GM-CSF, IL-6, IL-8, IL-11, and the chemokine regulated on activation, RANTES.¹⁶⁻¹⁹

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Abbreviations used

APRT:	Adenine phosphoribosyltransferase
ATCC:	American Type Culture Collection
CAT:	Chloramphenicol acetyltransferase
CO ₂ :	Carbon dioxide
CPE:	Cytopathic effect
DM:	Dexamethasone
DMSO:	Dimethyl sulfoxide
HC:	Hydrocortisone
IC ₅₀ :	50% Inhibitory concentration
ICAM-1:	Intercellular adhesion molecule-1
MEM:	Dulbecco's minimal essential medium
MF:	Mometasone furoate
MOI:	Multiplicity of infection
mRNA:	Messenger RNA
NF- κ B:	Nuclear factor- κ B
RT:	Reverse transcription
RV16:	Rhinovirus type 16
TCID ₅₀ :	Tissue culture infective dose 50%

The relevance of ICAM-1 to the inflammatory mechanisms of asthma has been clearly demonstrated in primate studies that showed that eosinophil infiltration into the airways correlated with epithelial ICAM-1 expression and that anti-ICAM-1 antibodies prevented both eosinophil influx and airway hyperreactivity.²⁰ Human studies have reported that high levels of ICAM-1 are expressed in asthmatic bronchial epithelium.^{21,22}

Given that respiratory epithelium is the primary site of rhinovirus infection in humans and because inflammatory cell infiltration of the epithelium is a common feature of rhinovirus-induced asthma, we wished to investigate the possible pharmacologic modulation of rhinovirus-induced epithelial cell ICAM-1 up-regulation.

Although corticosteroids are of undoubted benefit in asthma, their role in the treatment or prevention of virus-induced asthma exacerbations is still unclear.^{23,24} Mometasone furoate (MF) is one of the new generation of corticosteroids with negligible oral bioavailability, high topical potency, and low potential for systemic side effects.^{25,26} In vitro and in vivo studies have shown that MF is a potent inhibitor of production of several cytokines relevant to asthma and viral infection.^{27,28}

The aim of this study was therefore to investigate the effect of corticosteroids on rhinovirus-induced respiratory epithelial cell ICAM-1 surface expression and to investigate the capacity of MF to interfere with the pre-transcriptional mechanisms (messenger RNA [mRNA] induction and promoter up-regulation) of rhinovirus induction of ICAM-1.

MATERIAL AND METHODS

Cell culture

A549 cells, a type II respiratory epithelial cell line, were obtained from the American Type Culture Collection (ATCC), Rockville, Md, and Ohio HeLa (O'HeLa) cells, a cervical carcinoma epithelial cell line,²⁹ were obtained from the Medical Research

Council Common Cold Research Unit, Salisbury, United Kingdom. Cells were cultured at 37°C in 5% carbon dioxide (CO₂) in Eagle's minimal essential medium (MEM) supplemented with 4 mmol/L L-glutamine, 80 mg/mL gentamicin, and 10% FBS (Sigma, UK), and split weekly. Primary human bronchial epithelial cells were obtained by bronchial brushing from healthy patients, as previously described.⁸ Cells were removed from the brush by vigorous shaking and were disaggregated in bronchial epithelial cell medium (Clonetics, San Diego, Calif) containing 3 mmol/L dithiothreitol for 15 minutes. Cells were washed and plated onto collagen-coated 16-mm diameter culture wells and grown to confluence in bronchial epithelial cell medium. Cells were passaged into fresh flasks at a density of 10⁴ cells/cm² and used in the assays at passages 2 and 3. For experiments, 70% confluent cells were detached by 0.05% trypsin with 0.02% EDTA (Clonetics) and seeded in 12-well culture plates at 2 × 10³ cells per well. These cells are more than 95% cytokeratin 18-immunoreactive as assessed by immunofluorescence microscopy.

Viral stocks

Rhinovirus type 16 (RV16), which belongs to the major rhinovirus group, was obtained from Dr E. C. Dick, Madison, Wis. Neutralization testing with specific antiserum (ATCC) was used to confirm serotype identity.³⁰ Viral stocks were prepared by infection of sensitive cell monolayers (O'HeLa) until cytopathic effects (CPE) were fully developed. Cultures were then frozen at -70°C and then thawed, and clarified supernatants were frozen at -70°C.

Viral titration of aliquoted stocks was determined by exposing confluent monolayer of O'HeLa cells in 96-well plates to serial 10-fold dilutions of viral stock. CPE was evaluated after 5 days of culture in 4% MEM at 37°C in 5% CO₂ by visual assessment and by assessment of the continuity of the monolayer after removal of the medium, cell fixation in methanol, and staining with 0.1% crystal violet. Tissue culture infective dose 50% (TCID₅₀) per milliliter values were calculated. Virus at a multiplicity of infection (MOI) of 1 was used for all the experiments.

Flow cytometric analysis of ICAM-1 surface expression

Flow cytometry was used to quantify the level of ICAM-1 surface expression on resting and rhinovirus-infected A549 or primary bronchial epithelial cells in the presence or absence of corticosteroids. Briefly, 2 × 10⁵ cells were cultured in 12-well plates. When A549 cells were confluent, 10⁻⁶ to 10⁻¹² mol/L hydrocortisone (HC, Sigma), 10⁻⁶ to 10⁻¹² mol/L dexamethasone (DM, Sigma), 10⁻⁶ to 10⁻¹² mol/L MF (Schering Plough), or solvent alone (ethanol for HC and DM, dimethyl sulfoxide [DMSO] for MF, at a final concentration of 0.1% vol/vol) was added. Primary bronchial epithelial cells were pretreated with 10⁻⁷ mol/L DM, 10⁻⁷ mol/L MF, or solvent alone. Incubation continued for 16 hours before 8 hours of exposure to RV16 or medium alone. The duration of the exposure was chosen according to previous studies that had shown a maximal up-regulation of ICAM-1 expression after 8 hours of rhinovirus infection.⁸ Cells were thereafter washed 3 times with Ca⁺⁺- and Mg⁺⁺-free HBSS and treated for 10 minutes at 37°C with cell dissociation solution (Ca⁺⁺- and Mg⁺⁺-free HBSS containing 0.02% EDTA, Sigma) without trypsin and then removed from plates by repeated pipetting. More than 95% of cells were nonpermeable and viable as determined by trypan blue exclusion. A total of 5 × 10⁵ cells were then washed and resuspended in PBA (PBS, 1% BSA, 0.01% sodium azide) and incubated with saturating amounts of FITC antihuman ICAM-1 antibody (Serotec) or isotype-specific control antibody (Serotec) for 30 minutes at 4°C in the dark. Cells were washed twice in PBA. A total of 10⁴ cells were analyzed for

TABLE I. Mometasone furoate has no effect on ICAM-1 expression on A549 epithelial cells

Treatment	ICAM-1 mean fluorescence intensity
Control	12.3 ± 2.4
MF 10 ⁻¹² mol/L	10.6 ± 1.6
MF 10 ⁻¹¹ mol/L	12.2 ± 1.5
MF 10 ⁻¹⁰ mol/L	11.4 ± 1.4
MF 10 ⁻⁸ mol/L	9.4 ± 2.3
MF 10 ⁻⁷ mol/L	11.8 ± 1.9
MF 10 ⁻⁶ mol/L	10 ± 1.1
DMSO 0.1% vol/vol (vehicle)	11.1 ± 1.6

Data are expressed as mean ± SEM. There were no significant differences in ICAM-1 expression between any condition and control.

fluorescence by single-color flow cytometry on a FACScan analyzer (Becton Dickinson, San Jose, Calif). The mean fluorescence intensity was measured and calculated after subtraction of background staining. For each series of experiments, rhinovirus-induced ICAM-1 up-regulation was determined to be 100%, and the effect of steroid pretreatment was calculated as the percentage of it. The concentration inhibiting 50% (IC₅₀) of the observed ICAM-1 up-regulation was thereafter evaluated for each compound.

Reverse transcription-PCR analysis of ICAM-1 mRNA

Reverse transcription (RT)-PCR was used to evaluate ICAM-1 mRNA on resting and rhinovirus-infected A549 cells in the presence or absence of MF. Briefly, 5 × 10⁶ A549 cells were cultured in 100-mm plates. MF or solvent alone (DMSO, 0.1% vol/vol) was added, and incubation was continued for 16 hours before 8 hours of exposure to RV16 or medium alone, according to previous studies.⁸ Dose-response studies were performed with concentrations of MF from 10⁻¹² to 10⁻⁷ mol/L. Cells were harvested, and RNA was extracted with Trizol (Gibco BRL). One microgram of total RNA was used for a quantitative estimation of ICAM-1 mRNA expression by specific RT-PCR as previously described.⁸

ICAM-1 promoter activation

Reporter gene construct. An ICAM-1 promoter–chloramphenicol acetyltransferase (CAT) construct containing the full-length sequence (–1160 bp) of the ICAM-1 5'-flanking region linked to the coding region of the CAT reporter gene was used for transfection.

Cell transfection and CAT assay. A549 cells were transfected with the reporter construct (10 µg) by the calcium phosphate coprecipitation technique as previously described.⁸ Transfected cells were cultured in 10% MEM overnight. MF 10⁻⁷ mol/L, or solvent alone (DMSO, 0.1% vol/vol) were added, and incubation continued for 16 hours before the addition of RV16 or medium alone. Twenty-four hours later, cells were harvested, proteins extracted, and protein-equivalent aliquots assayed for CAT activity. The assay was performed at 37°C for 60 minutes in a reaction mixture of 1 mmol/L acetyl coenzyme A (Pharmacia) and 0.1 µCi of carbon 14-labeled chloramphenicol. Acetylated and nonacetylated forms of chloramphenicol were resolved by thin-layer chromatography, visualized by autoradiography, and measured on a scintillation counter. CAT activity was expressed as percentage conversion of chloramphenicol into its acetylated derivatives.

Rhinovirus replication assessment

Rhinovirus replication in the presence of corticosteroids was evaluated by performing titration assays as described above. Briefly, 10-fold dilutions of viral stock were incubated with confluent monolayers of O'HeLa cells in 96-well plates, in the presence or the absence of 10⁻⁶ mol/L HC, DM, or MF for 16 hours before infection. The cells were incubated in 4% MEM at 37°C in 5% CO₂ for 5 days, fixed in methanol, and stained with 0.1% crystal violet. The CPE was evaluated by visual assessment and by assessment of the continuity of the monolayer. For each experiment TCID₅₀ per milliliter values were calculated in the samples with and without corticosteroids.

Statistical analysis

Data were expressed as mean ± SEM, and comparison between groups was performed by paired and unpaired Student *t* tests. All experiments were carried out at least 4 times.

RESULTS

MF inhibits RV16-induced ICAM-1 up-regulation on A549 respiratory epithelial cells

A549 epithelial cells were cultured to confluence in 24-well tissue culture plates and incubated for 8 hours with RV16 in the presence or the absence of 10⁻⁶ to 10⁻¹² mol/L MF.

ICAM-1 was constitutively expressed on A549 cells (mean fluorescence intensity 12.3 ± 2.5), and its basal expression was not affected by vehicle or by MF at any of the tested concentrations (Table I).

As previously reported,⁸ 8 hours of RV16 infection, in the presence of the vehicle alone, significantly increased surface epithelial expression to 5.4 ± 1.2-fold over control uninfected cells (*P* < .01). Pretreatment with MF inhibited rhinovirus ICAM-1 induction in a dose-response fashion (Fig 1), with an IC₅₀ of 10⁻¹¹ mol/L.

Comparison of MF with other corticosteroids

A549 epithelial cells were cultured to confluence in 24-well tissue culture plates and incubated for 8 hours with RV16 in the presence or the absence of 10⁻⁶ to 10⁻¹² mol/L HC, DM, or vehicle alone.

ICAM-1 basal expression was not affected by vehicle alone, HC, or DM at any of the tested concentrations (data not shown).

As observed for MF, pretreatment with HC or DM inhibited rhinovirus ICAM-1 induction in a dose-response fashion, with IC₅₀s of 10⁻¹⁰ and 10⁻¹¹ mol/L for HC (Fig 2, A) and DM (Fig 2, B), respectively.

Corticosteroids inhibit RV16-induced ICAM-1 up-regulation on primary bronchial epithelial cells

Primary bronchial epithelial cells were cultured to confluence in 24-well tissue culture plates and incubated for 8 hours with RV16 in the presence or absence of 10⁻⁷ mol/L MF or 10⁻⁷ mol/L DM.

ICAM-1 was constitutively expressed on primary bronchial epithelial cells (mean fluorescence intensity 9.7 ± 2.1), and its basal expression was not affected by vehicle or by 10⁻⁷ mol/L MF or 10⁻⁷ mol/L DM (mean

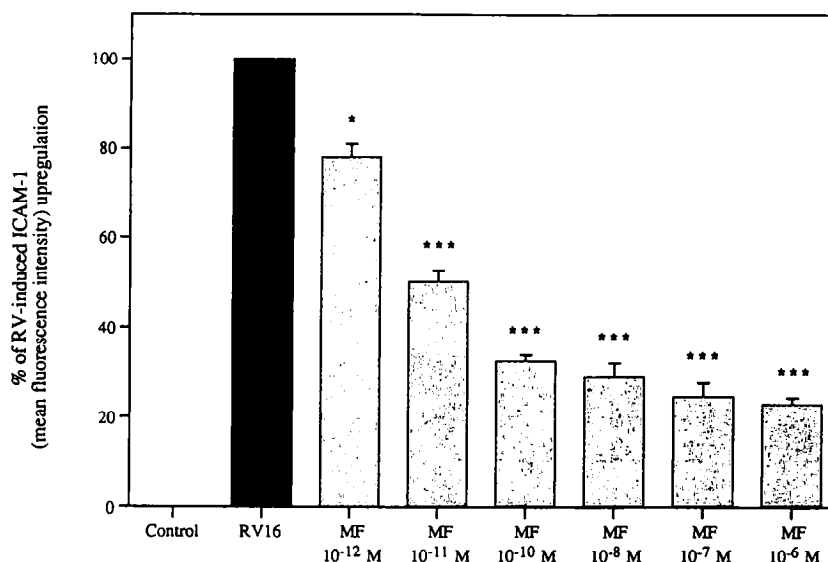


FIG 1. Effect of MF on RV16-induced ICAM-1 surface expression on A549 pulmonary epithelial cells. Cells were incubated with vehicle or MF at doses from 10^{-12} to 10^{-6} mol/L for 16 hours and then exposed to medium alone or RV16 for 8 hours. ICAM-1 expression was measured by flow cytometry and values were normalized as percent of rhinovirus-induced ICAM-1 up-regulation in non-pretreated samples. Data are mean $\pm$ SEM of at least 4 separate experiments. Asterisk, $P < .05$; three asterisks, $P < .001$ versus samples not pretreated with MF.

fluorescence intensity 9.4 ± 2.3 , 8.7 ± 1.8 , and 9.8 ± 2.7 , respectively).

As previously reported,⁸ 8 hours of RV16 infection, in the presence of the vehicle alone, significantly increased surface epithelial expression to 7.3 ± 1.9 -fold over control uninfected cells ($P < .01$). As observed in Fig 3, pretreatment of primary bronchial epithelial cells with 10^{-7} mol/L MF or 10^{-7} mol/L DM significantly inhibited rhinovirus-induced ICAM-1 up-regulation to a degree similar to that observed in the A549 cell line (Figs 1 and 2, B).

MF inhibits rhinovirus ICAM-1 mRNA induction

Having demonstrated that MF strongly suppressed rhinovirus-induced ICAM-1 surface protein expression, we investigated the effect of MF on rhinovirus-induced ICAM-1 mRNA expression.

A549 epithelial cells were cultured in 100-mm tissue culture plates. Confluent cells were exposed to medium alone or RV16 for 8 hours in the presence of either vehicle or MF (10^{-12} to 10^{-7} mol/L). Uniform processing was confirmed by adenine phosphoribosyltransferase (APRT) RT-PCR performed in parallel. Densities of APRT demonstrated less than 10% variability among all samples, and ICAM-1 densities were normalized according to the APRT values.

Control A549 cells contain very low levels of ICAM-1 mRNA. RV16 strongly induced ICAM-1 mRNA expression after 8 hours of infection, in accordance with our previous findings.⁸ MF inhibited rhinovirus induction of ICAM-1 mRNA in a dose-dependent manner (Table II), confirming that MF-induced suppression of ICAM-1 surface protein was likely a result of inhibition

TABLE II. MF pretreatment inhibits rhinovirus induction of ICAM-1 mRNA expression in A549

Conditions	ICAM-1 mRNA expression (ICAM-1/APRT densitometric ratio) (arbitrary units)	
	Control	RV16
Control	0	
RV16		1.83 ± 0.08
MF 10^{-12} /RV16		1.69 ± 0.09
MF 10^{-10} /RV16		0.34 ± 0.04
MF 10^{-7} /RV16		0.11 ± 0.03

Data are expressed as mean $\pm$ SEM.

of mRNA transcriptional up-regulation. A representative experiment is depicted in Fig 4, where a clear increase in levels of ICAM-1 mRNA was detectable after RV16 infection (lane 2), whereas MF pretreatment (10^{-12} mol/L, lane 3; 10^{-10} mol/L, lane 4; and 10^{-7} mol/L, lane 5) resulted in a dose-dependent inhibition of ICAM-1 mRNA induction.

MF inhibits RV16-induced ICAM-1 promoter activation

We have previously demonstrated that NF- κ B-mediated promoter activation is critically involved in induction of ICAM-1 by rhinovirus infection.⁸ Corticosteroids are known to inhibit NF- κ B-mediated transcriptional regulation^{31,32}; we therefore carried out studies to determine whether MF inhibited ICAM-1 promoter activation induced by rhinovirus infection. A549 cells were transfected with a construct containing the CAT reporter gene whose transcription was regulated by the full-length ICAM-1 promoter. As shown in Fig 5, lanes 3 and 4,

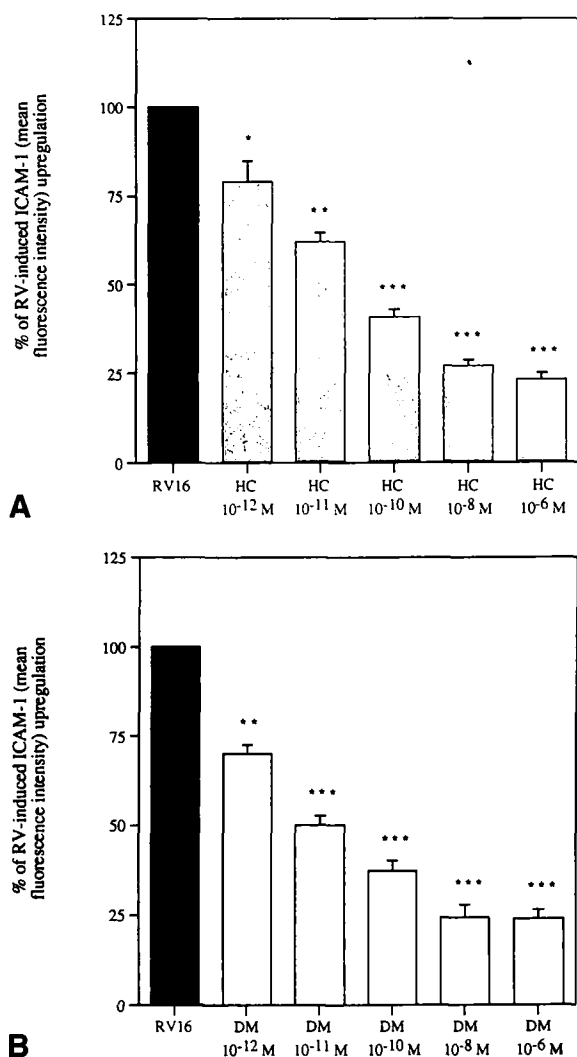


FIG 2. Effects of HC or DM on RV16-induced ICAM-1 surface expression on A549 pulmonary epithelial cells. **A**, Cells were incubated with vehicle or HC at doses from 10^{-12} to 10^{-6} mol/L for 16 hours and then exposed to medium alone or RV16 for 8 hours. **B**, Cells were incubated with vehicle or DM at doses from 10^{-12} to 10^{-6} mol/L for 16 hours and then exposed to medium alone or RV16 for 8 hours. ICAM-1 expression was measured by flow cytometry, and values normalized as percent of rhinovirus-induced ICAM-1 up-regulation in control non-pretreated samples. Data are mean $\pm$ SEM of at least 4 separate experiments. Asterisk, $P < .05$; two asterisks, $P < .01$; three asterisks, $P < .001$ versus samples not pretreated with corticosteroids.

RV16-induced ICAM-1 promoter activity compared with media-inoculated cells (acetylation $1.6\% \pm 0.5\%$ and $41.2\% \pm 5.2\%$ for control and infected cells, respectively, $P < .001$). The presence of vehicle alone did not modify the rhinovirus-induced ICAM-1 promoter activation previously described.⁸ MF 10^{-7} mol/L pretreatment completely abrogated rhinovirus induction of ICAM-1 promoter activity (acetylation $1.7\% \pm 0.5\%$ and $1.8\% \pm 0.6\%$ for control and infected cells after MF pretreatment, respectively, Fig 5, lanes 1 and 2). These results confirm that MF inhibits rhinovirus-induced ICAM-1

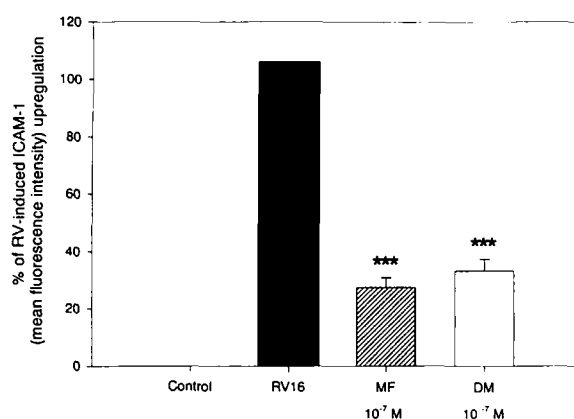


FIG 3. Effects of MF or DM on RV16-induced ICAM-1 surface expression on primary bronchial epithelial cells. Cells were incubated with vehicle, 10^{-7} mol/L MF, or 10^{-7} mol/L DM for 16 hours and then exposed to medium alone or RV16 for 8 hours. ICAM-1 expression was measured by flow cytometry, and values were normalized as percent of RV-induced ICAM-1 up-regulation in control non-pretreated samples. Data are mean $\pm$ SEM of at least 4 separate experiments. Three asterisks, $P < .001$ versus samples not pretreated with corticosteroids.

promoter activation. The upper panel of Fig 5 shows a representative autoradiograph demonstrating the effect of MF on ICAM-1 promoter activation by rhinovirus infection.

Effect of corticosteroids on rhinovirus replication and infectivity

We have demonstrated that MF inhibits rhinovirus-induced ICAM-1 surface protein and mRNA expression and promoter activation. We also showed that other corticosteroids similarly inhibit rhinovirus-induced ICAM-1 expression. Although there is no evidence to our knowledge that rhinovirus infection is inhibited by corticosteroids and although we found no evidence of MF suppression of constitutive ICAM-1 expression in A549 cells (Table I), it has been shown that corticosteroid pretreatment of cells down-regulates ICAM-1 (the rhinovirus receptor) expression in bronchial epithelial cells.³³ It is therefore plausible that this effect of corticosteroids might result in inhibition of viral replication. We therefore wished to confirm that the observed effects were not simply related to corticosteroid inhibition of rhinovirus replication. To evaluate whether corticosteroids affect rhinovirus replication, titration assays were performed to assess replication in a sensitive cell line. O'HeLa cells were inoculated with RV16 at an MOI of 1 in the presence or absence of 10^{-6} mol/L HC, 10^{-6} mol/L DM, or 10^{-6} mol/L MF for 16 hours before infection. These concentrations were chosen because they were the highest used in the earlier studies. As shown in Table III, at 5 days there was no difference in TCID₅₀ per milliliter mean values between corticosteroid pretreated and control samples. Further, no difference was found in the progression of CPE as assessed by visual inspection on a daily basis (data not shown). These data demonstrate

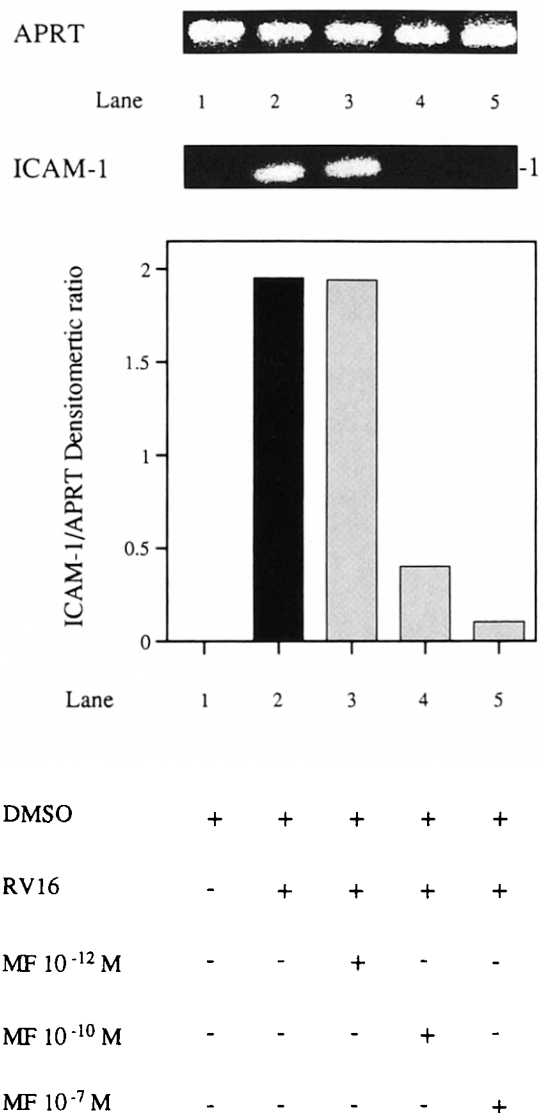


FIG 4. Effect of MF on RV16-induced ICAM-1 mRNA expression in A549 pulmonary epithelial cells. Representative (of at least 4 separate experiments) RT-PCR analysis for ICAM-1 and APRT (house-keeping gene) expression in A549 respiratory epithelial cells uninfected (lane 1) and incubated with RV16 at an MOI of 1 in the absence or presence of MF at indicated concentrations for 16 hours. RNA was harvested for analysis at 8 hours of infection. *Top*, Ethidium bromide-stained gel electrophoreses of products of RT-PCR for APRT and ICAM-1; *bottom*, ICAM-1/APRT ratio determined by densitometric analysis.

that corticosteroids have no inhibitory effect on rhinoviral replication in a sensitive cell line.

DISCUSSION

ICAM-1 is the receptor of 90% of the rhinoviruses and is an adhesion protein that has a central role in inflammatory cell recruitment to the lower airway after rhinovirus infection. In previous studies, we have provided evidence that rhinovirus infection of respiratory epithelial cells increases cell surface expression of ICAM-1

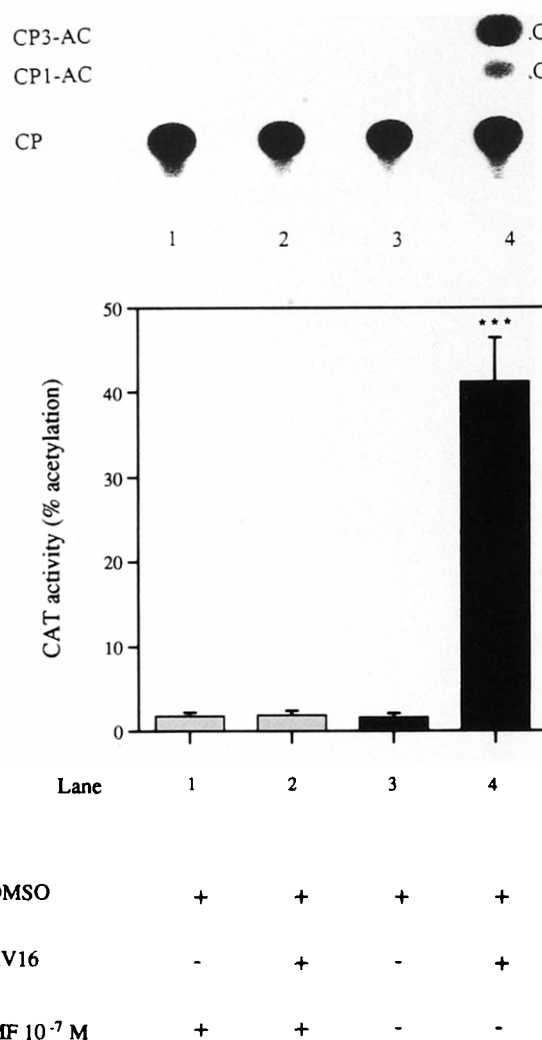


FIG 5. Inhibition by MF of RV16-induced ICAM-1 promoter activation in A549 pulmonary epithelial cells. A549 cells were transiently transfected with a full-length (~ 1160) ICAM-1 promoter-CAT construct and incubated with medium alone or RV16 at an MOI of 1 in the presence or absence of MF 10^{-7} mol/L for 16 hours. Epithelial cells were harvested after 24 hours of infection and CAT activity in protein equivalent cell lysates was assessed as described in Material and methods. *Top*, Representative autoradiograph of CAT assay; *bottom*, ICAM-1 promoter activation expressed as percent of chloramphenicol acetylation. Data are mean $\pm$ SEM of at least 5 separate experiments. *Three asterisks*, $P < .001$ versus control uninfected cells. *CP*, Unacetylated chloramphenicol; *CP1-AC* and *CP3-AC*, acetylated forms of chloramphenicol.

through NF- κ B. In the current study we have investigated the effects of corticosteroids on rhinovirus-induced ICAM-1 expression. We have demonstrated that corticosteroids inhibited rhinovirus induction of surface protein expression of ICAM-1 on both primary bronchial epithelial cells and the type II respiratory epithelial cell line A549. On A549 cells we demonstrated that corticosteroids act in a dose-dependent manner and that MF is as effective as DM and that both are more potent than HC.

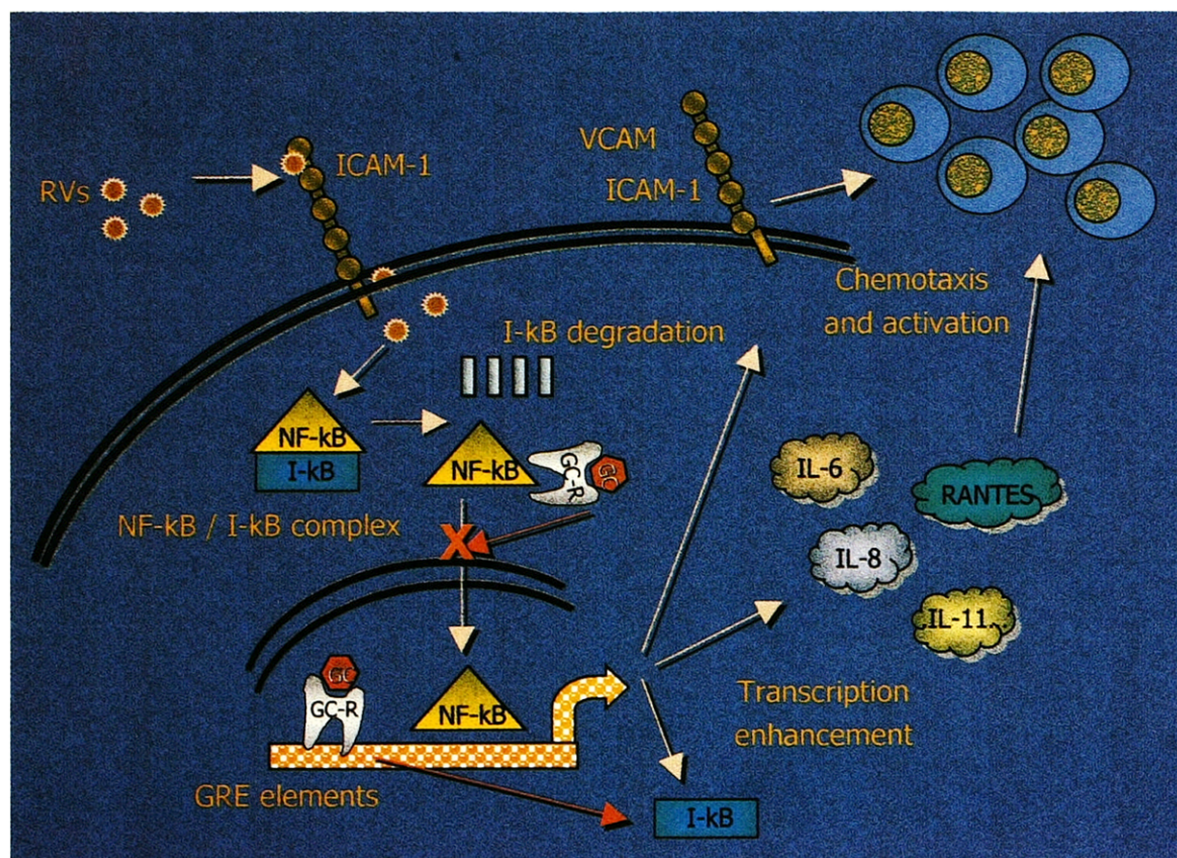


FIG 6. Possible effects of glucocorticosteroids (GC) on rhinovirus (RV)-induced inflammation in the airway. Schematic representation of rhinovirus-induced proinflammatory mechanisms in the airway, including induction of ICAM-1, vascular cell adhesion molecule (VCAM), and cytokine production by transactivation of promoter NF- κ B binding sites. Corticosteroids interfere by having first complexed with their cytoplasmic receptors (GC-R), (1) by interacting directly with NF- κ B/Rel family proteins to inhibit their DNA binding and induction of proinflammatory molecules (red cross) and (2) by binding to glucocorticoid response elements (GRE) and either repressing proinflammatory molecule gene transcription or inducing promoter activation of anti-inflammatory molecules such as I- κ B and thus reducing the amount of NF- κ B available for translocation into the nucleus (red arrow).

TABLE III. Corticosteroid treatment has no effect on rhinovirus replication in O'HeLa cells

Treatment	Virus titer ($\log_{10}$)
Control	6.94 $\pm$ 0.21
HC 10 ⁻⁶ mol/L	6.83 $\pm$ 0.23
DM 10 ⁻⁶ mol/L	7.03 $\pm$ 0.12
MF 10 ⁻⁶ mol/L	6.90 $\pm$ 0.17

Data are expressed as mean $\pm$ SEM. There were no significant differences in virus titer between any condition and control.

We then investigated the effect of MF on ICAM-1 mRNA induction and promoter activity in A549 cells. We found that MF exerts a dose-dependent inhibition of rhinovirus-induced ICAM-1 mRNA expression and completely suppressed rhinovirus-induced ICAM-1 promoter activation. Finally, we demonstrated that corticosteroids do not inhibit rhinovirus replication in a sensitive cell line.

Corticosteroids have been potent and effective drugs for the treatment of asthma and allergic diseases since their discovery decades ago. However, recent studies have reported that they are not satisfactorily effective for virus-induced asthma exacerbations, either as prophylactic or as acute-phase treatment.^{23,24} Corticosteroids have multiple, well-characterized means by which they interfere with asthmatic airway inflammation, including alteration of cell trafficking of lymphocytes and inflammatory cells such as eosinophils, blockade of mediator release from inflammatory cells, and inhibition of cytokine production.^{28,34,35} In vitro studies have also shown the down-regulatory effect of corticosteroids on epithelial surface ICAM-1 expression, whereas inhaled corticosteroids have been recently reported to decrease ICAM-1 expression on bronchoalveolar lavage fluid cells obtained from subjects with mild asthma.^{33,36}

The ICAM-1 promoter contains several potential transcription factor binding sites, several of which have been implicated in induction of ICAM-1 gene transcription in

response to various proinflammatory stimuli.^{37,38} In particular, the -187/-178 NF- κ B site on the ICAM-1 promoter has been recently described as a necessary and sufficient element for rhinovirus induction of ICAM-1 promoter activity⁸ and as a site of corticosteroid repression on that promoter.³⁹

Corticosteroids are known to inhibit a number of possible pathways involved in rhinovirus-induced inflammation by suppressing both ICAM-1 and cytokine induction. The proposed mechanisms involved are summarized in Fig 6.

The control experiments performed on a rhinovirus-sensitive cell line (O'HeLa) permitted evaluation of the effect of the drugs on rhinovirus replication. We chose to perform these experiments in O'HeLa cells to examine the effects of corticosteroids independently from the up-regulatory effect of rhinovirus on its own receptor (ICAM-1) observed in respiratory epithelial cells.⁸ Indeed, ICAM-1 surface expression decreases on rhinovirus-infected O'HeLa cells in parallel with the appearance and the severity of CPEs (data not shown). These experiments demonstrated that the highest concentration of corticosteroids used in the respiratory epithelial cell experiments had no effect on rhinovirus replication and infectivity in this sensitive cell line (Table III). These data suggest that corticosteroids are also unlikely to affect rhinovirus replication in respiratory epithelium independently of their effect on ICAM-1 expression, which we have demonstrated occurs at the level of inhibition of rhinovirus-induced ICAM-1 promoter activity.

Given the lymphocyte and eosinophil infiltration observed after experimental rhinovirus infection, its role as rhinovirus receptor, and the fact that rhinovirus infection up-regulates respiratory epithelial ICAM-1 expression, ICAM-1 is likely to play a critical role in promoting lower airway inflammation in virus-induced asthma. Our demonstration that corticosteroids strongly inhibit this induction suggests that inhaled corticosteroids with increased potency and reduced systemic effects are an attractive option for treatment of virus-induced asthma exacerbations. We observed that MF is as potent as DM and 1 log more potent than HC in modulating rhinovirus-induced ICAM-1 up-regulation (IC₅₀ of 10⁻¹⁰, 10⁻¹¹, and 10⁻¹¹ mol/L for HC, DM, and MF, respectively). This observation is relevant from a therapeutic point of view because it suggests a possible intervention strategy by the use of topical corticosteroids as potent as systemic corticosteroids but without systemic side effects. This approach should therefore be assessed clinically in the prevention or treatment of virus-induced asthma exacerbations.

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