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Induction of B7-H1 and B7-DC expression on airway epithelial cells by the Toll-like receptor 3 agonist double-stranded RNA and human rhinovirus infection: *In vivo* and *in vitro* studies

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Abstract

Background—T-cell infiltration of the epithelium is a key feature of chronic rhinosinusitis and asthma. Viral infections are an important cause of disease exacerbations. We have found virus-induced expression of T cell-interacting ligands, B7 homolog costimulatory molecules, on airway epithelium.

Objective—We tested the ability of human rhinovirus (HRV) 16 and double-stranded RNA (dsRNA) to alter the expression of B7 homologs on human airway epithelial cells.

Methods—BEAS2B and primary human airway epithelial cells were exposed *in vitro* to dsRNA (25 µg/mL) or HRV-16, and then expression of cell-surface protein and mRNA for B7 homologs was assessed by means of flow cytometry and real-time PCR, respectively. Additionally, human subjects were infected with HRV-16 *in vivo*, and mRNA for B7 homologs was assessed by means of real-time PCR in fresh nasal epithelial cell scrapings obtained before and daily up to 4 days after infection.

Results—dsRNA exposure of BEAS2B and human primary bronchial epithelial cells resulted in increased levels of cell-surface and mRNA expression of B7-H1 and B7-DC but not B7-H2 or B7-H3. Exposure of primary cells to HRV-16 resulted in induction of cell-surface expression of B7-H1 and B7-DC. Pretreatment with fluticasone propionate failed to suppress the induction of B7-H1 and B7-DC. Nasal scrapings taken at the time of peak symptom scores (3 days) after infection of 6 human subjects with HRV-16 displayed selective induction of levels of mRNA for B7-H1 and B7-DC.

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Clinical implications: Rhinovirus induction of epithelial B7 homologs might influence the development of adaptive immune responses in the airways.

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Conclusion—These data show that HRV-16 infection or exposure to dsRNA induces epithelial B7-H1 and B7-DC.

Keywords

B7 homologs; costimulatory ligands; cell-surface molecules; human airway epithelial cells; rhinovirus infection; double-stranded RNA

Human rhinovirus (HRV) infections are strong inducers of inflammatory diseases of the airways. Rhinovirus is the major cause of the common cold and the most common pathogen associated with exacerbations of asthma and sinusitis in both adults and children.^{1–3} Although rhinovirus can infect monocytes, macrophages, and fibroblasts, the major site of HRV infection is thought to be the airway epithelial cell. In contrast to infection of leukocytes and fibroblasts, the virus resides and replicates within the epithelial cell without compromising host cell viability.⁴ Instead of a cytotoxic response, the epithelial cell responds to infection by elaborating host defense and inflammatory mediators, which in turn contribute to recruitment and activation of inflammatory cells, such as neutrophils, eosinophils, and T cells.^{5–7} T cells recruited by the immediate antiviral response of the host epithelial cells are thought to be predominantly type 1 T cells, which elaborate T_H1-type cytokines, including TNF- α and IFN- γ . These and other mediators in turn function to activate epithelial cells and amplify the inflammatory response. Thus there appears to be significant paracrine crosstalk between T cells and epithelial cells in orchestrating the inflammatory response.

T-cell infiltration of the epithelium is a common feature of chronic rhinosinusitis (CRS) with nasal polyps and asthma.^{8,9} The majority of T cells in nasal polyp tissue are activated T cells, which express CD45RO, CD25, and CD69.¹⁰ These intra-epithelial T cells of nasal polyps express the potent ligand for epithelial E-cadherin, $\alpha_E\beta_7$ integrin (CD103).⁸ Airway epithelial cells also express cell-surface molecules associated with antigen presentation, including MHC class I¹¹ and class II,¹⁰ CD40,^{12,13} and the ligand for T-cell surface receptor lymphocyte function-associated antigen 1, intercellular adhesion molecule 1.¹⁴ Thus in addition to direct physical contact between the T cells and epithelial cells, there are several ligand/receptor molecules expressed on airway epithelial cells, which can bind to respective receptor/ligand complements on T cells. Taken together, these data support the notion that the airway epithelium might interact directly with T cells and regulate their function. Whether epithelial cells play a direct role in adaptive immune responses remains uncertain.

Several studies from one group have shown that epithelial cells have the capacity to present antigens to and to stimulate T cells *in vitro*.^{15–17} However, the mechanisms underlying this effect have been unclear because epithelial cells lack the expression of costimulatory molecules B7.1 and B7.2, which are required for optimal activation of T cells. We have recently reported, however, that airway epithelial cells constitutively express levels of homologs of B7 costimulatory ligands: B7-H1, B7-H2, B7-H3, and B7-DC.^{18,19} We demonstrated that the proinflammatory cytokines TNF- α and IFN- γ or IFN- γ alone selectively increased B7-H1 and B7-DC but not B7-H2 and B7-H3 expression in both BEAS2B and primary human bronchial epithelial cells *in vitro*. In coculture experiments of purified human T cells with BEAS2B cells, we also saw that inhibition of B7-H1 and B7-DC with blocking antibody resulted in enhancement of IFN- γ expression from T cells. Thus in our studies B7-H1 and B7-DC on airway epithelial cells functioned to regulate T-cell activation by inhibiting T-cell production of IFN- γ .

In our prior studies, we found increased expression of B7 homologs in sinus tissue from patients with CRS.¹⁸ We thus sought to extend these studies to examine whether relevant innate immune stimuli implicated in exacerbations of asthma and CRS could modulate expression of B7

costimulatory ligands of airway epithelial cells. To do this, we examined the effect of double-stranded RNA (dsRNA) exposure and viral infection on induction of B7 homolog expression on human airway epithelial cells *in vitro* and by infection of human subject volunteers *in vivo*.

METHODS

Rationale

To test the hypothesis that mucosal inflammation triggered by viral dsRNA might regulate B7 homolog expression, we examined the effect of synthetic dsRNA and anti-inflammatory glucocorticoid on B7 homolog expression in the immortalized BEAS2B airway epithelial cell line. Next we examined the ability of dsRNA to induce B7 homolog expression in cultured human primary bronchial epithelial cells (PBECs). Because HRV-16 is known to be a key trigger of exacerbations of CRS and asthma, we examined whether HRV-16 infection would reproduce the effects of dsRNA as a stimulus to induce expression of B7 homologs in both PBECs and cultured human primary nasal epithelial cells (PNECs). Only first-passage cells were used for all studies involving primary human airway epithelial cells. Lastly, we extended the studies to examine the effect of experimental rhinovirus infection of human subjects on B7 homolog expression from nasal airway epithelial cells.

Collection of human PNECs and PBECs

For *in vitro* experiments, primary airway epithelial cells were obtained from nasal and bronchial sources. PNECs were collected from the inferior nasal turbinate by means of curettage with a cytology brush (Wampole, Harrisburg, Pa), as previously described.¹⁸ Care was taken to take 10 uniform passes of mucosal scrapings. The specimen was placed in Ca²⁺- and Mg²⁺-free Ham's F12/Dulbecco's modified Eagle's medium (DMEM) containing penicillin (100 U/mL), streptomycin (100 U/mL), and amphotericin B (1 µg/mL), and L-glutamine (GIBCO-BRL, Gaithersburg, Md) for cell culture. Each nasal scraping specimen yielded approximately 1 to 2 × 10⁶ cells, of which more than 90% were confirmed to be epithelial cells by using Wright's stain and cytokeratin immunofluorescence. PBECs were harvested by means of protease digestion of fresh human lung from organ donors, as previously described.²⁰ All procedures were performed under JHMIRB-approved human subject research protocols.

Culture of epithelial cells and stimulation with dsRNA

The BEAS-2B cell line, derived from human bronchial epithelium transformed by an adenovirus 12-SV40 virus, was kindly supplied by Dr Curtis Harris.²¹ BEAS-2B cells, PNECs, and PBECs were cultured in 6-well plates to 80% confluence, as previously described, by using DMEM/Ham's F12 with 5% heat-inactivated FBS, penicillin (100 U/mL), streptomycin (100 U/mL), amphotericin B (1 µg/mL), and L-glutamine (2 mmol/L).¹⁸ The cells were subsequently exposed to synthetic dsRNA (poly IC; Sigma-Aldrich, St Louis, Mo) at the indicated concentration for 24 hours and then washed 3 times with 0.05% trypsin/0.53 mmol/L ethylenediamine tetraacetic acid in Hanks' balanced salt solution (HBSS) or with 0.02% ethylenediamine tetraacetic acid in HBSS for analysis of mRNA expression or for flow cytometry, respectively. Generally, the cell recovery was approximately 5 to 8 million cells per 6-well plate. The viability of both BEAS-2B cells and primary epithelial cells at the time of cell harvest was assessed by means of erythrosin B staining or propidium iodide exclusion and was consistently greater than 95% of the cells harvested. For experiments assessing the effect of steroids, cells were pretreated with 10⁻⁷ mol/L fluticasone propionate or appropriately diluted dimethyl sulfoxide vehicle control for 4 hours before stimulus exposure. Fluticasone propionate was again included in the medium after stimulus exposure.

***In vitro* viral infection**

HRV-16 was propagated in WI-38 cells and purified to remove ribosomes and soluble factors of WI-38 origin by means of centrifugation through sucrose, according to published methods.²² For infection, monolayers of epithelial cells (70% to 80% confluent) were washed 3 times with HBSS. HRV-16 was added to the cells at concentrations of 5×10^4 tissue culture infective dose (TCID₅₀) U/mL HBSS. The cells were incubated with the virus at 34°C for 3 hours and washed 3 times with DMEM, and then fresh DMEM (2.5 mL/well of a 6-well plate) was added to the cells. Cells were harvested for studies 72 hours later, and cell viability of monolayers was confirmed by means of light microscopy.

Flow cytometry, isolation of mRNA, and quantitative Taqman real-time PCR

Flow cytometric analysis, isolation of mRNA, and mRNA analysis of B7 homologs was performed by using specific antibodies, Taqman probes and primers, and methods as previously described.¹⁸

***In vivo* viral infection clinical protocol**

Safety-tested HRV-16 stock containing 1500 TCID₅₀ U/0.01 mL was prepared as recommended by assaying for the absence of bacteria, fungi, viruses, or mycoplasma.^{23,24} Informed consent was obtained from 6 nonsmoking, nonasthmatic healthy study volunteers between the ages of 23 and 49 years (mean age, 35 years; 1 female subject) under a JHMRB-approved study protocol. All subjects had no cold symptoms in the previous 6 weeks and negative serum-neutralizing antibodies to HRV-16 before recruitment. Subjects were inoculated twice in 1 hour with 0.250 mL per nostril of HRV-16 stock, as described previously, to a total dose of 1000 TCID₅₀ HRV-16 and demonstrated seropositive conversion after infection.^{25,26} Assessment of symptom scores and collection of nasal lavage fluid were performed before infection on day 0 and days 1 to 7 after infection. Eight symptoms (sneezing, stuffy nose, runny nose sore throat, cough, headache, chills or fever, and malaise) were recorded daily on a scale of 0 (none) to 3 (severe). Nasal lavage was performed with 10 mL of lactated Ringers solution and stored at -80°C for subsequent analysis of viral titers and real-time PCR for rhinovirus. Nasal scrapings were performed on days 0 to 3 and obtained by means of Rhinoprobe curettage of 2 separate areas of the inferior turbinate, as previously described.^{25,26} The RNA was isolated by using the Qiagen RNeasy kit (Qiagen, Valencia, Calif).

Statistical analysis

All data are expressed as means $\pm$ SEMs unless otherwise indicated. The statistical significance of the effect of dsRNA, rhinovirus, and fluticasone propionate on cell-surface expression of B7 homologs was determined by using ANOVA. When a significant difference between groups was observed, *post-hoc* analysis was performed by using the Fisher least significant difference test. Differences were considered significant for *P* values of less than .05. Statistical significance of the effect of dsRNA, rhinovirus, and fluticasone propionate on mRNA expression of B7 homologs was determined by using the Wilcoxon signed-rank test for nonparametric analysis of paired samples.

RESULTS

Effect of dsRNA on B7 homolog expression in BEAS2B cells

The data in Fig 1 (upper panel) show that 24-hour exposure to dsRNA increased cell-surface expression of B7-H1 and B7-DC but not B7-H2 or B7-H3 in BEAS2B cells. mRNA analysis by means of real-time PCR supported these findings in that both B7-H1 and B7-DC mRNA levels were selectively induced in response to 24-hour exposure to dsRNA (Fig 1, lower panel). In contrast, dsRNA and fluticasone had little or no effect on levels of cell-surface expression

and mRNA expression of B7-H2 and B7-H3. The selective induction of cell-surface B7-H1 and B7-DC by dsRNA was dose dependent, as shown in Fig 2. Exposure to the potent glucocorticoid fluticasone propionate (10^{-7} mol/L) resulted in inhibition of the induction of both B7-H1 and B7-DC cell-surface protein and mRNA expression by dsRNA. Fluticasone did not significantly reduce the baseline cell-surface protein expression of B7-H1 and B7-DC. We failed to detect expression of B7-H4 in BEAS2B cells by means of either flow cytometric analysis or real-time PCR mRNA analysis in unstimulated control cells or after stimulation with dsRNA (data not shown).

Effect of dsRNA on B7 homolog expression in PBECs

As shown in Fig 3, dsRNA exposure of PBECs for 24 hours significantly induced B7-H1 and B7-DC cell-surface expression but had no effect on B7-H2 and B7-H3 expression, which is similar to the results seen in BEAS2B cells. mRNA analysis also demonstrated that dsRNA selectively induced B7-H1 and B7-DC but had no effect on B7-H2 and B7-H3. Thus dsRNA induction of B7-H1 and B7-DC was observed in both transformed bronchial epithelial cell lines and primary cultured bronchial epithelial cells from human subjects. However, in contrast to findings in the BEAS2B cells, fluticasone propionate pretreatment of PBECs had no effect on the induction of B7-H1 and B7-DC by dsRNA and had no effect on constitutive baseline expression. In addition, fluticasone propionate had no effect on B7-H2 or B7-H3 expression under either stimulated or baseline conditions. The inability of *in vitro* steroid exposure to inhibit the induction of B7-H1 and B7-DC in primary cell cultures paralleled the response obtained previously with IFN- γ as a stimulus.¹⁸ Consistent with findings in BEASB cells, B7-H4 was not detected by means of either mRNA analysis or flow cytometric analysis of cell-surface protein in PBECs (data not shown).

Effect of *in vitro* rhinovirus infection on B7 homolog expression in primary human airway epithelial cells

Fig 4 shows the results of *in vitro* HRV-16 infection on B7 homolog expression in human primary epithelial cells derived from both the bronchus and the nose. The results show that both B7-H1 and B7-DC were selectively induced in both PBECs (upper panel: by 90% and 110%, respectively) and PNECs (lower panel: by 140% and 400%, respectively) after 72 hours exposure of primary airway epithelial cells to HRV-16. There was no effect of viral exposure of primary airway epithelial cells on B7-H2 or B7-H3 expression. Pretreatment with fluticasone propionate did not inhibit the induction of B7-H1 or B7-DC by HRV-16 and had no effect on baseline or stimulated levels of B7-H2 and B7-H3. These results demonstrate that the effect of *in vitro* rhinovirus infection mirrored the effect of dsRNA in its ability to selectively induce B7-H1 and B7-DC expression in human primary airway epithelium. In addition, similar to dsRNA as a stimulus, fluticasone propionate did not inhibit the induction of B7 homologs by HRV-16 on both types of the primary cells.

Effect of *in vivo* rhinovirus infection on B7 homolog expression in human nasal epithelial cells

Based on the positive study results with rhinovirus *in vitro*, we extended the studies to examine whether experimental rhinovirus infection of human subjects would result in induction of B7 homologs on nasal airway epithelial cells. From the nasal scraping specimens, we isolated mRNA for assays of B7 homologs and rhinovirus. Quantitation of HRV-16 from the nasal scrapings by means of real-time PCR correlated highly with the results of a cytotoxicity bioassay of nasal lavage fluid in WI-38 cells ($r = 0.88$, $P = .0001$), as shown in Fig 5. Viral titers peaked on day 2 of infection and correlated with peak symptom scores ($P = .01$). The results demonstrate that clinically symptomatic rhinovirus infection was achieved. By using this protocol, the time course of the effect of *in vivo* HRV-16 infection on B7 homolog mRNA

expression from fresh epithelial cells derived from nasal scrapings was examined. Fig 6 demonstrates a 25-fold mean increase in B7-H1 expression and a 10-fold mean increase in B7-DC mRNA expression, which peaked on day 2 of infection, during the time of peak symptoms. The *in vivo* induction reached statistical significance on day 3 of infection, with an 8.5-fold increase in B7-H1 and a 3.2-fold increase in B7-DC mRNA expression. In contrast, there was no effect of *in vivo* HRV-16 infection on expression of B7-H2 and B7-DC mRNA. Thus the selective induction of B7-H1 and B7-DC on epithelium observed *in vitro* with the Toll-like receptor 3 agonist, HRV-16 or its replication product dsRNA, is recapitulated *in vivo* with rhinovirus challenge of human subjects.

DISCUSSION

Interactions between T cells and epithelial cells are thought to be important in the pathogenesis of asthma and CRS. Products of both T_H1- and T_H2-activated T cells are known to stimulate proinflammatory cytokine and chemokine production in epithelial cells. IL-4 and IL-13 secreted from T_H2-activated T cells are known to stimulate signal transducer and activator of transcription 6-dependent secretion of eotaxin and other CC chemokines in epithelial cells.^{27,28} Additionally, IL-1 and TNF- α are known to induce epithelial cell production of IL-6, IL-8, and GM-CSF.²⁹ There is a growing literature to indicate that epithelial cells reciprocate to control T-cell responses. IFN- γ -induced protein of 10 kDa (IP-10) is a non-ELR CXC chemokine produced by epithelial cells that serves as a selective chemoattractant for activated type 1 T cells.³⁰ Recently, the airway epithelium has been identified as a major source of thymic stromal lymphopoietin, a potent mediator of T_H2 inflammation that stimulates myeloid dendritic cells to promote the differentiation of T cells into T_H2 cells that produce IL-4 and IL-13.^{31–33} However, whether epithelial cells can control T-cell function by means of direct interaction and the role of T cell-interacting molecules found on the surface of airway epithelial cells is less clear. Our laboratory has previously shown that IFN- γ can induce selective expression of B7-H1 and B7-DC on airway epithelial cells, suggesting that IFN- γ might serve to activate epithelial cells for interaction with T cells *in vivo*.¹⁸ Upper respiratory tract infections are thought to be a key trigger of exacerbations of asthma and CRS that contribute to disease progression. Thus we sought to examine the effect of rhinovirus infection on epithelial expression of T cell-interacting molecules, the B7 homolog family of ligands.

In vitro exposure of airway epithelial cells to dsRNA resulted in induction of both cell-surface expression and mRNA expression of B7-H1 and B7-DC but not B7H2 or B7-H3. This was observed in both the BEAS2B cell line and in PBECs. The pattern of response to dsRNA was similar to that seen with IFN- γ in both BEAS2B cells and PBECs, as reported previously.¹⁸

Our laboratory and many others have shown that the BEAS2B cell line is a useful model for examination of human airway epithelial cell function.^{34,35} In many instances the cellular responses reflect those of cultured primary human airway epithelial cells, including steroid responses. For example, when epithelial GM-CSF expression was examined, both BEAS2B cells and PBECs have been shown to demonstrate similar responses to glucocorticoids *in vitro*, in that both cell types demonstrate exquisite sensitivity to the influence of glucocorticoids.^{36–38} However, in our studies there was a clear discrepancy in steroid responsiveness of B7-H1 and B7-DC expression between cultured primary human airway epithelial cells and an immortalized cell line. The potent glucocorticoid fluticasone inhibited the *in vitro* induction of B7-H1 and B7-DC by dsRNA in BEAS2B cells. In contrast to the BEAS2B cell line, fluticasone pretreatment failed to inhibit dsRNA induction of B7-H1 and B7-DC specifically in human primary airway epithelial cells. The failure of glucocorticoids to suppress B7-H1 and B7-DC induction was demonstrated in human airway epithelium from 2 distinct (bronchial and nasal) sources, indicating that the inability of glucocorticoids to suppress B7-H1 and B7-DC during stimulation or at baseline is a common feature of human

primary airway epithelial cells. Interestingly, the same pattern of steroid responsiveness in BEAS2B cells and unresponsiveness in primary airway epithelial cells was observed previously when IFN- γ was used as a stimulus for induction of B7-H1 and B7-DC.¹⁸ The discrepancy in the steroid responsiveness between cultured primary human airway epithelial cells and an immortalized cell line is unclear. This points to the inherent differences between immortalized cell lines and primary cells and the need to examine both in the context of the specific biomarker in question.

When the direct effect of rhinovirus infection was examined, we found that *in vitro* HRV-16 infection mirrored the effect of its surrogate dsRNA in its ability to selectively induce B7-H1 and B7-DC in primary airway epithelial cells. Resistance to glucocorticoids was again demonstrated because fluticasone pretreatment of primary airway epithelial cells resulted in no effect of induction of B7 homologs by HRV-16. Although *in vitro* studies show that glucocorticoids can affect rhinovirus replication, cytokine release, and upregulation of intercellular adhesion molecule 1,^{39–41} the efficacy of glucocorticoids in the treatment of rhinovirus-induced exacerbations of asthma is lacking.^{42–44} Thus our observations are consistent with the notion of the lack of efficacy of glucocorticoids to suppress rhinovirus-induced epithelial cell priming. To examine the relevance of rhinovirus induction of B7 homologs in human disease, we studied the effect of experimental HRV infection *in vivo*. We found that HRV-16 infection of human subjects resulted in selective induction of B7-H1 and B7-DC mRNA levels from nasal epithelial cell scrapings taken during the period of peak infection, corroborating results seen with our *in vitro* studies.

B7-H1 and B7-DC are putative inhibitory costimulatory molecules. Ligation of these ligands with their counterreceptor, programmed death receptor-1 (PD-1), can result in inhibition of T- and B-cell responses. More recently, PD-1 activity has been shown to be required for the termination of the late phase of allergic inflammation.⁴⁵ Overexpression of B7-H1 in a mouse Langerhans dendritic cell line suppressed T-cell activation by inhibiting IFN- γ production from activated T cells and inhibiting T-cell proliferation.⁴⁶ *In vivo* administration of dendritic cells carrying hapten and overexpressing B7-H1 into mice resulted in a hapten-specific desensitization response.⁴⁶ Activation of B7-DC on dendritic cells by pentameric antibody crosslinking was shown to result in inhibition of the allergic inflammatory response in ovalbumin-sensitized mice.^{47,48} Thus B7-H1 and B7-DC appear to play a role in controlling allergic responses by inhibiting T-cell activation. In the present studies, induction of the inhibitory costimulatory molecules B7-H1 and B7-DC on epithelial cells occurred as a response to viral infection, a major trigger of inflammation. Whether viral induction of epithelial B7-H1 and B7-DC results in control of T-cell activation remains to be examined. Furthermore, whether this response represents an attempt by the host epithelium to curtail perpetuation of virally induced inflammatory signals remains to be explored. The consequences of T-cell engagement of these inhibitory costimulatory molecules on epithelial cells might result in either a beneficial or detrimental effect on the host, depending on the specific function of the T cell being engaged. These studies demonstrate that the epithelial cell has an ability to respond to the Toll-like receptor 3 agonists, dsRNA and HRV-16, by expressing surface molecules with the potential to interact with and suppress the activity of T cells. Viral induction of B7-H1 and B7-DC might represent a pathway by which innate immune mediators can stimulate epithelial cells to regulate adaptive immune responses.

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Abbreviations used

CRS	Chronic rhinosinusitis
DMEM	Dulbecco modified Eagle medium
dsRNA	Double-stranded RNA
HBSS	Hanks' balanced salt solution
HRV	Human rhinovirus
JHMIRB	Johns Hopkins Medicine Institutional Review Board
PBEC	Primary bronchial epithelial cell
PNEC	Primary nasal epithelial cell
TCID ₅₀	Tissue culture infective dose

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FIG 1.

Induction of BEAS2B cell-surface B7-H1 and B7-DC by dsRNA is inhibited by the potent glucocorticoid fluticasone. BEAS2B cells ($n = 6$) were cultured in control medium or 25 $\mu\text{g}/\text{mL}$ dsRNA for 24 hours, with and without fluticasone (FP; 10^{-7} mol/L). Flow cytometric analysis (expressed as mean fluorescence intensity [MFI], *upper panel*) and mRNA analysis (expressed as fold induction of mRNA over control conditions, *lower panel*) was performed as previously described.¹⁸ * $P < .01$, ** $P < .005$, and *** $P < .001$.

FIG 2.

Dose-dependent induction of BEAS2B cell-surface B7-H1 and B7-DC by dsRNA. BEAS2B cells ($n = 3$) were cultured in control medium or indicated concentrations of dsRNA for 24 hours, with and without fluticasone (10^{-7} mol/L). Flow cytometric analysis (expressed as mean fluorescence intensity [MFI]) for B7-H1 (*left panel*) and B7-DC (*right panel*) was performed as previously described.¹⁸ * $P < .05$ compared with control condition without dsRNA. + $P < .05$ compared with condition with dsRNA alone. ++ $P < .08$ compared with condition with dsRNA alone.

FIG 3.

dsRNA induces cell-surface and mRNA levels of B7-H1 and B7-DC on PBECs. PBECs were cultured in control medium or dsRNA (25 $\mu\text{g}/\text{mL}$) for 24 hours with and without fluticasone (FP; 10^{-7} mol/L). Flow cytometric analysis (expressed as mean fluorescence intensity [MFI], upper panel, $n = 5$) and mRNA analysis (expressed as fold induction of mRNA over control conditions, lower panel, $n = 4$) was performed as previously described.¹⁸ * $P < .05$, ** $P < .01$, *** $P < .005$, and **** $P < .001$.

FIG 4.

In vitro HRV-16 infection induces cell-surface B7-H1 and B7-DC expression on primary airway epithelial cells. PBECs (*upper panel*, n = 6) and PNECs (*lower panel*, n = 6) were cultured in control medium or exposed to HRV-16 with and without fluticasone (FP; 10^{-7} mol/L). Flow cytometric analysis for B7 homologs was performed as previously described.¹⁸ Data are expressed as mean fluorescence intensity (MFI). * $P < .01$, ** $P < .05$, and *** $P < .005$.

FIG 5.

In vivo HRV-16 challenge results in development of symptoms of upper respiratory tract viral infection. Human subjects ($n = 6$) were intranasally inoculated with 1000 TCID₅₀ of safety-tested HRV-16 stock on day 1. Symptom scores and nasal lavage specimens were obtained on day 0 before inoculation and days 1 to 7, as described in the Methods section. Nasal lavage specimens were assayed for viral titers by means of both cytotoxicity bioassay in WI-38 cells or real-time RT-PCR for HRV-16, and the results were expressed as log TCID₅₀.

FIG 6.

In vivo HRV-16 challenge increases levels of mRNA for B7-H1 and B7-DC in nasal epithelial scrapings. Human subjects (n = 6) were infected with HRV-16, as described in the Methods section. The results are expressed as fold change comparing nasal scraping samples taken after infection with samples taken before infection. * $P < .05$ compared with pre-infection state.