Expression of Programmed Death–1 Ligand (PD-L) 1, PD-L2, B7-H3, and Inducible Costimulator Ligand on Human Respiratory Tract Epithelial Cells and Regulation by Respiratory Syncytial Virus and Type 1 and 2 Cytokines

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Background. Respiratory syncytial virus (RSV) is associated with wheezing illness, and infections can occur repeatedly throughout life. We hypothesized that RSV infection of respiratory tract epithelial cells up-regulates B7 molecules that regulate memory immune responses and that type 1 and 2 cytokines differentially modulate this induction.

Methods. We used flow-cytometric analysis to investigate programmed death–1 ligand (PD-L) 1, PD-L2, B7-H3, and inducible costimulatory ligand (ICOS-L) expression on tracheal (NCI-H292), bronchial (BEAS-2B), and alveolar (A549) epithelial cells; regulation of this expression by RSV, interferon (IFN)–γ, and interleukin (IL)–4; and the effects of IFN-γ and IL-4 on RSV-induced expression of these molecules.

Results. B7-H3 was strongly expressed, PD-L1 and ICOS-L were moderately expressed, and PD-L2 was weakly expressed on unstimulated tracheal, bronchial, and alveolar epithelial cells. RSV infection up-regulated PD-L1, PD-L2, and B7-H3 expression on all cells and ICOS-L expression on bronchial and alveolar epithelial cells. IL-4 treatment alone had no effect, whereas IFN-γ treatment alone increased PD-L1 and PD-L2 expression on all cells and decreased B7-H3 expression on bronchial and alveolar epithelial cells. On RSV-infected alveolar epithelial cells, IFN-γ treatment increased PD-L1 and PD-L2 expression and decreased B7-H3 and ICOS-L expression, and IL-4 treatment increased PD-L2 and B7-H3 expression and decreased ICOS-L expression.

Conclusions. Respiratory tract epithelial cells express a wide range of B7 molecules. RSV infection increases their expression, and this expression is differentially regulated by IFN-γ and IL-4. These processes may be involved in decreasing T cell antiviral immune responses to RSV and in RSV-associated wheezing.

Respiratory syncytial virus (RSV) is the most common cause of hospitalization for severe lower respiratory tract infection in infants [1], and it causes severe respiratory tract illness in older immunodeficient children [2] and the elderly [3]. Bronchiolitis caused by RSV during infancy is associated with the later development of asthma [4], and RSV is implicated in exacerbations of asthma and chronic obstructive pulmonary disease (COPD) [5–8]. Protective immunity to reinfection with RSV is incomplete and of short duration: previously infected infants [9] and adults [10] remain susceptible to reinfection with both antigenically related viruses and the identical virus strain. Failure to completely clear RSV infection is suggested by studies in mice [11], guinea pigs [12], rats [13], and

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of COPD in humans [14]. The mechanisms for the deficient protective memory immune response to RSV infection are not known, although it has been shown that RSV infection suppresses CD8+ T cell adaptive immune responses [15].

Ligation of costimulatory molecules such as programmed death-1 (PD-1) ligand 1 (PD-L1), PD-L2, B7-H3, and inducible costimulator (ICOS) ligand (ICOS-L) with their receptors on T cells play an important role in regulating T cell responses [16–18], PD-L1 (also known as B7-H1) and PD-L2 (also known as B7-H2 and B7-DC) are ligands to the PD-1 receptor and to another, unidentified receptor on activated T cells [16–18]. Investigators initially identified the PD-1 receptor through subtractive hybridization studies, using a T cell hybridoma undergoing programmed cell death (hence its name), but subsequent studies have not shown a direct role for PD-1 in cell death [16–18].

PD-1 ligand expression can be rapidly up-regulated on various cells and tissues. PD-L1 and PD-L2 expression on murine macrophages is up-regulated by both Th1 cytokines (such as interferon [IFN]–γ) and Th2 cytokines (such as interleukin [IL]–4) [19], but, on human macrophages, IFN-γ up-regulates PD-L1 expression and, in contrast, IL-4 up-regulates PD-L2 expression [20]. In terms of their function, initial studies reported a T cell activatory function for PD-1/PD-1 ligand pathways, but more recent data suggest that PD-1/PD-L1 pathways function as negative regulatory pathways during both primary and, more potently, secondary T cell responses [21]. The controversial data on the inhibitory or activatory role of PD-L1 and PD-L2 on T cell functions might be explained by the existence of 2 T cell receptors (inhibitory receptor PD-1 and a putative, unknown costimulatory receptor), and, for PD-L1, preliminary data suggest that low levels of PD-L1 augment T cell responses and that high levels inhibit them [22].

B7-H3 was initially identified as a costimulatory molecule for human activated T cell responses through an unidentified receptor [23]. B7-H3 is normally undetectable on resting hematopoietic cells and healthy organs, but the expression of B7-H3 can be up-regulated by various stimuli [23]. The immunological function of B7-H3 is also controversial: some studies have found that human B7-H3 has T cell stimulatory function [23], but, in vivo, the absence of B7-H3 augments Th1 (but not Th2 or antiviral CD8+ T cell responses), suggesting that B7-H3 may be an inhibitory molecule for Th1 responses [24].

ICOS-L (also known as B7RP-1, B7h, B7-H2, and LICOS) [16, 18] binds to its receptor ICOS, which is expressed on activated T cells. ICOS-L is constitutively expressed on B cells, macrophages, and dendritic cells (antigen-presenting cells [APCs]) and is up-regulated on APCs and some nonlymphoid tissues, including lung tissue, by inflammatory stimuli. Most evidence suggests that ICOS/ICOS-L is a positive costimulatory pathway through which T cell help is delivered to B cells and cytokine production by Th2 cells is enhanced [16, 25]. In a respiratory tract hyperreactivity model, ICOS has been shown to be important in T cell regulatory function and to inhibit Th2 responses [26], and ICOS–ICOS-L interactions inhibit both Th1 and Th2 responses in some circumstances [27].

Together, the available data suggest that the up-regulation of these molecules might inhibit type 1 (by PD-L1, B7-H3, and ICOS-L) and also type 2 (by PD-L2 and ICOS-L) T cell immune responses. In lung epithelial cells, the alteration of the balance between inhibitory and costimulatory molecules in favor of inhibitory molecules could play a role in decreasing antiviral memory immune responses.

We have previously shown that respiratory tract epithelial cells express the costimulatory molecules intercellular adhesion molecule 1, B7-1, and B7-2 and that their expression is up-regulated by respiratory tract virus infection [28]. Recent studies of respiratory tract epithelial cells have shown that both unstimulated BEAS-2B cells and primary bronchial epithelial cells (PBECs) spontaneously and strongly express ICOS-L mRNA and that BEAS-2B cells also express mRNA for PD-L1, PD-L2, and B7-H3 [29]. Flow-cytometric analysis has shown that ICOS-L is constitutively expressed on BEAS-2B cells and PBECs and is down-regulated by tumor necrosis factor (TNF)–α, IFN-γ, IL-4, and various combinations of these cytokines [29]. Another research group has found that PD-L1 and ICOS-L are present on respiratory tract epithelial cells and that their surface expression increases after treatment with IFN-γ [30].

Recent data have suggested that PD-1/PD-L1 interactions may contribute to the functional inactivation of virus-specific CD8+ T cells during chronic viral infection [31, 32]. We therefore hypothesized that a mechanism of the deficient memory immune response to RSV could involve RSV-induced expression of inhibitory costimulatory molecules on respiratory tract epithelial cells, thereby leading to defective RSV-specific memory immune responses. We then further hypothesized that a mechanism of the association between RSV and wheezing could be differential regulation by type 1 and 2 cytokines of the RSV-induced expression of the B7 family of molecules—which are implicated in regulating Th1/Th2 immune responses—on respiratory tract epithelial cells. In the present study, we investigated whether tracheal, bronchial, and alveolar epithelial cells constitutively express PD-L1, PD-L2, B7-H3, and ICOS-L and whether this expression is up-regulated by RSV infection or type 1 and 2 cytokines. Finally, we investigated whether RSV-induced PD-L1, PD-L2, B7-H3, and ICOS-L expression on respiratory tract epithelial cells was differentially regulated by IFN-γ and IL-4.

MATERIALS AND METHODS

**Cell culture.** Human RSV strain A2 was used (gift from Prof. P. J. Openshaw, Imperial College London, London, United King-
or cells/well (A549 cells) in medium containing 10% FCS. In MFI (of at least 4 separate experiments), com-
mean of stimulation experiments were expressed as the fold induction by secondary staining with RPE-conjugated antibody). Results for unstimulated cells were expressed as the mean fluorescence intensity (MFI) measured after subtracting the baseline fluorescence of unstimulated cells. An MOI of 1 was used for all experiments, except where indicated otherwise.

Tracheal (NCI-H292), bronchial (BEAS-2B), and alveolar (A549) epithelial cell lines were obtained from the European Collection of Cell Cultures. Cells were grown in RPMI 1640 (NCI-H292 and BEAS-2B cells) or Eagle MEM (A549 cells) supplemented with Glutamax (Invitrogen) and 10% fetal calf serum (FCS; Invitrogen) and buffered with 0.075% sodium bicarbonate (Invitrogen) and 0.25 mmol/L HEPES (Invitrogen). Cells were seeded in 12-well plates at concentrations of 3 × 10^5 cells/well (NCI-H292 cells), 1.7 × 10^5 cells/well (BEAS-2B cells), or 1 × 10^5 cells/well (A549 cells) in medium containing 10% FCS. After 24 h, the medium was replaced with medium containing 2% FCS. Because preliminary experiments indicated that there were no differences in the expression of costimulatory molecules between unstimulated cells at time 0 and unstimulated cells after 24 h of culture in 2% FCS (data not shown), the MFI of cells harvested at 24 h (48 h for B7-H3) after culture was investigated by flow-cytometric analysis. B7-H3 was strongly expressed, PD-L1 and ICOS-L were moderately expressed, and PD-L2 was weakly expressed on unstimulated NCI-H292, BEAS-2B, and A549 cells (figure 1 and table 1).

**RESULTS**

**Constitutive Expression of PD-L1, PD-L2, B7-H3, and ICOS-L on Respiratory Tract Epithelial Cells**

Because preliminary experiments indicated that there were no differences in the expression of costimulatory molecules between unstimulated cells at time 0 and unstimulated cells after 24 h of culture in 2% FCS (data not shown), the MFI of cells harvested at 24 h (48 h for B7-H3) after culture was investigated by flow-cytometric analysis. B7-H3 was strongly expressed, PD-L1 and ICOS-L were moderately expressed, and PD-L2 was weakly expressed on unstimulated NCI-H292, BEAS-2B, and A549 cells (figure 1 and table 1).

**Up-Regulation of B7 Molecule Expression on Respiratory Tract Epithelial Cells by RSV Infection**

To determine the time course and virus specificity of the modulation of PD-L1, PD-L2, B7-H3, and ICOS-L expression by RSV infection, costimulatory molecule expression was determined by flow-cytometric analysis of respiratory tract epithelial cells exposed to either RSV or UV-inactivated RSV in time-course and dose-response experiments (figure 2).

**Time-course experiments.** NCI-H292, BEAS-2B, and A549 cells were cultured for up to 48 h with RSV (1 MOI) and harvested, and flow cytometry was performed. RSV infection up-regulated PD-L1 (at 24 h and 48 h, P < .05), PD-L2 (at 24 h, P < .05), and B7-H3 (at 48 h, P < .05) expression on NCI-H292 cells (figure 2A). The capacity of RSV infection to up-regulate costimulatory molecule expression was confirmed on BEAS-2B cells: RSV infection significantly up-regulated PD-L1 (at 24 h, P < .05; at 48 h, P < .01), PD-L2 (at 24 and 48 h, P < .05), B7-H3 (at 24 and 48 h, P < .05), and ICOS-L (at 24 h, P < .05) expression (figure 2B).

PD-L1 and PD-L2 expression on A549 cells was also significantly up-regulated by RSV infection, starting at 24 h (for both, P < .001) and continuing at 48 h (for both, P < .001) (figure 2C). However, the increase in PD-L1 expression was ~10 times more than that in PD-L2 expression at both time points. RSV infection of A549 cells also up-regulated B7-H3 expression at 48 h (P < .05) and ICOS-L expression at 4, 24, and 48 h (for all, P < .001) (figure 2C).

**Dose-response experiments.** We performed a dose-response study in which A549 cells were exposed to increasing doses of RSV (0.1, 0.3, and 1 MOI) or to UV-inactivated RSV, cells were harvested at 24 and 48 h, and flow cytometry was performed. Figure 2D shows PD-L1, PD-L2, and ICOS-L expression at 24 h and B7-H3 expression at 48 h. PD-L1 and PD-L2 expression on A549 cells was significantly up-regulated...
Programmed death–1 ligand (PD-L) 1, PD-L2, B7-H3, and inducible costimulator ligand (ICOS-L) expression on NCI-H292, BEAS-2B, and A549 cells. Cells cultured in medium only were harvested at 24 h for detection of PD-L1, PD-L2, and ICOS-L expression and at 48 h for detection of B7-H3 expression. Cells were stained with primary mouse antibody against human PD-L1, PD-L2, B7-H3, or ICOS-L and secondary R-phycoerythrin–conjugated goat anti–mouse IgG. Data are representative of 6 experiments. Shown are histograms of cells stained with negative isotype control antibody (A) or specific antibody (B).

by increasing doses of RSV (for all doses, P < .001) (figure 2D). For all RSV doses, the increase in PD-L1 expression was almost 10 times more than that in PD-L2 expression. RSV infection of A549 cells up-regulated B7-H3 and ICOS-L expression in a dose-dependent manner, because higher doses of RSV induced higher levels of expression (figure 2D).

Virus specificity. The capacity of RSV to amplify costimulatory molecule expression was virus replication specific in that UV-inactivated RSV had no effect on the expression of these molecules on A549 cells (figure 2D).

IFN-γ and IL-4 Induction of B7 Molecule Expression on Respiratory Tract Epithelial Cells

We next determined the effect that the type 1 cytokine IFN-γ and the type 2 cytokine IL-4 had on B7 molecule expression on NCI-H292, BEAS-2B, and A549 cells (figure 3).

IFN-γ time-course experiments. NCI-H292, BEAS-2B, and A549 cells were cultured for up to 48 h with IFN-γ (50 ng/mL) and harvested, and flow cytometry was performed. On NCI-H292 cells, IFN-γ treatment up-regulated PD-L1 and PD-L2 expression for up to 48 h (at 24 and 48 h, P < .05) and did not significantly alter B7-H3 and ICOS-L expression (figure 3A). On BEAS-2B cells, IFN-γ treatment up-regulated PD-L1 expression for up to 48 h (at 4 h, P < .05; at 24 and 48 h, P < .001), up-regulated PD-L2 expression only at 48 h (P < .05), slightly down-regulated B7-H3 expression at 24 h (P < .05), and did not significantly alter ICOS-L expression (figure 3B). On A549 cells, IFN-γ treatment induced PD-L1 expression (at 24 and 48 h, P < .001) and PD-L2 expression (at 24 and 48 h, P < .05) (figure 3C). However, PD-L1 expression was 2–3-fold greater at each time point, compared with PD-L2 expression. IFN-γ treatment slightly down-regulated B7-H3 expression at 24 h (P < .05) (figure 3C) and did not significantly alter ICOS-L expression (figure 3C).

IFN-γ dose-response experiments. We performed a dose-response study in which A549 cells were exposed to increasing concentrations of IFN-γ (1, 5, 50, and 100 ng/mL), cells were harvested at 24 and 48 h, and flow cytometry was performed.
DISCUSSION

We have demonstrated that tracheal, bronchial, and alveolar epithelial cells express the surface B7 molecules PD-L1, PD-L2, B7-H3, and ICOS-L and that their expression is up-regulated by RSV infection. RSV-induced expression of these molecules on A549 cells was differentially regulated by IFN-γ or IL-4 at different time points. At 24 h after RSV infection, IFN-γ treatment increased the RSV-induced expression of both PD-L1 and PD-L2 (PD-L1 more than PD-L2) and decreased the RSV-induced expression of ICOS-L. At 48 h after RSV infection, the up-regulation of RSV-induced PD-L1 and PD-L2 expression and the down-regulation of RSV-induced ICOS-L expression by IFN-γ treatment persisted, and IFN-γ treatment also down-regulated RSV-induced B7-H3 expression. IL-4 treatment of A549 cells up-regulated PD-L2 expression at 24 h but not at 48 h. At 48 h, unlike the effect of IFN-γ treatment, IL-4 treatment up-regulated RSV-induced B7-H3 expression; but, like the effect of IFN-γ treatment, IL-4 treatment down-regulated RSV-induced ICOS-L expression.

Severe RSV infection during infancy is associated with the development of asthma, and RSV infection is associated with asthma and COPD exacerbations in children and adults [4–8]. Frequent reinfection with RSV and the finding of latent infection with RSV in animal models and in patients with stable COPD suggests a deficient memory immune response to RSV [9, 10, 14]. We believe that the balance between costimulatory and coinhibitory signals from surface molecules expressed on nonprofessional APCs, such as respiratory tract epithelial cells, might determine the strength of the T cell activation. PD-L1, PD-L2, B7-H3, and ICOS-L are recently described surface molecules of the B7 family that are reported to have inhibitory effects on T cell responses; PD-L1 and B7-H3 inhibit Th1 re-

Table 1. Constitutive expression of B7 molecules on respiratory tract epithelial cells.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>PD-L1</th>
<th>PD-L2</th>
<th>B7-H3</th>
<th>ICOS-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H292</td>
<td>76.66 ± 18.08</td>
<td>4.90 ± 1.03</td>
<td>509.76 ± 107.90</td>
<td>14.51 ± 3.06</td>
</tr>
<tr>
<td>BEAS-2B</td>
<td>52.23 ± 12.34</td>
<td>18.16 ± 6.35</td>
<td>657.69 ± 143.03</td>
<td>41.30 ± 8.74</td>
</tr>
<tr>
<td>A549</td>
<td>8.19 ± 2.10</td>
<td>6.73 ± 1.76</td>
<td>418.52 ± 120.55</td>
<td>27.54 ± 7.19</td>
</tr>
</tbody>
</table>

NOTE. Data are the mean fluorescence intensity (± SE) from 6 experiments. ICOS-L, inducible costimulator ligand; PD-L1, programmed death-1 ligand 1; PD-L2, programmed death-1 ligand 2.

Figure 3D shows PD-L1, PD-L2, and ICOS-L expression at 24 h and B7-H3 expression at 48 h. IFN-γ treatment increased PD-L1 expression in a dose-dependent manner (for 10 ng/mL, P < .05; for 50 and 100 ng/mL, P < .001) (figure 3D). The increase in PD-L2 expression on A549 cells was also dose dependent and was significant only for higher doses (for 50 and 100 ng/mL, P < .01) (figure 3D). At each dose, IFN-γ treatment increased PD-L1 expression almost 10 times more than PD-L2 expression on A549 cells. B7-H3 expression was slightly decreased by treatment with all concentrations of IFN-γ (figure 3D). The increase in ICOS-L expression on A549 cells was also dose dependent and was significant for only the highest dose (100 ng/mL; P < .01) (figure 3D). IL-4 treatment at different concentrations (2.5, 5, and 10 ng/mL) and at different time points (4, 24, and 48 h) did not significantly alter the expression of any B7 molecule on NCI-H292, BEAS-2B, or A549 cells (P not significant; data not shown).

IFN-γ and IL-4 Modulation of RSV-Induced B7 Molecule Expression on A549 Cells

Finally, we examined the effect that cytokines had on RSV-induced expression of B7 molecules implicated in regulating Th1/Th2 immunity. A549 cells were cultured with RSV and either with or without IFN-γ (50 ng/mL) or IL-4 (10 ng/mL), cells were harvested at 24 and 48 h, and flow cytometry was performed. Figure 4 shows PD-L1, PD-L2, and ICOS-L expression at 24 h and B7-H3 expression at 48 h.

IFN-γ treatment up-regulated RSV-induced PD-L1 expression at 24 h (P < .001) (figure 4A) and 48 h (fold induction with RSV or RSV and IFN-γ, 34 ± 3.4 vs. 99 ± 11.3; P < .001; data not shown) and RSV-induced PD-L2 expression at 24 h (P < .05) (figure 4B) and, to a greater degree, at 48 h (fold induction with RSV or RSV and IFN-γ, 3.34 ± 0.27 vs. 10.35 ± 2.31; P < .001; data not shown). However, IFN-γ treatment down-regulated RSV-induced B7-H3 expression at 48 h (P < .05) (figure 4C) and RSV-induced ICOS-L expression at 24 h (P < .01) (figure 4D) and 48 h (fold induction with RSV or RSV and IFN-γ, 1.65 ± 0.06 vs. 1.28 ± 0.02; P < .05; data not shown).

IL-4 treatment did not modulate RSV-induced PD-L1 expression up to 48 h (figure 4A and data not shown) but did up-regulate RSV-induced PD-L2 expression at 24 h (P < .001) (figure 4B), although this effect disappeared at 48 h (data not shown). IL-4 treatment also weakly up-regulated RSV-induced B7-H3 expression at 48 h (P < .05) (figure 4C) and down-regulated RSV-induced ICOS-L expression at 48 h (fold induction with RSV or RSV and IL-4, 1.65 ± 0.06 vs. 1.28 ± 0.06; P < .01; data not shown).
Figure 2. Respiratory syncytial virus (RSV)–induced programmed death–1 ligand (PD-L) 1, PD-L2, B7-H3, and inducible costimulator ligand (ICOS-L) expression on NCI-H292, BEAS-2B, and A549 cells. Shown are the time courses of RSV induction of PD-L1, PD-L2, B7-H3, and ICOS-L expression on NCI-H292 (A), BEAS-2B (B), and A549 (C) cells. Cells cultured in medium only (white squares) or medium and RSV (1 MOI; black triangles) were harvested at 4, 24, and 48 h and stained for PD-L1, PD-L2, B7-H3, or ICOS-L expression. Results are expressed as the fold induction in mean fluorescence intensity (MFI), compared with the MFI of cells treated with medium only. Data are means ± SEs of 4 experiments. In a separate experiment (D), A549 cells were treated with RSV at different concentrations (0.1, 0.3, or 1 MOI; black bars), with UV-inactivated RSV (hatched bars), or with medium only (m; white bars) and then were harvested at 24 h (48 h for B7-H3); examined by indirect immunofluorescence staining for PD-L1, PD-L2, B7-H3, or ICOS-L expression; and assessed by flow-cytometric analysis. Data are means ± SEs of 4 experiments. *P < .05; **P < .01; ***P < .001.

Responses, PD-L2 inhibits Th2 responses, and ICOS-L inhibits both Th1 and Th2 responses [17, 21, 24, 26, 27], acting via inhibitory receptors.

Although the significance of B7 homologue expression on respiratory tract epithelial cells is not established, it has been hypothesized that PD-L, B7-H3, and ICOS-L expression by respiratory tract epithelium may play an important role in maintaining or regulating the activation of antigen-specific lymphocytes that have migrated into the respiratory tract and may play a crucial role in regulating immune responses [30]. In the present study, we have confirmed the previously published data on the constitutive expression of PD-L1 and ICOS-L on the surface of respiratory tract epithelial cells [29, 30]. In addition, we also found very strong B7-H3 expression and weak PD-L2 expression on unstimulated tracheal, bronchial, and alveolar epithelial cells. Our data support the hypothesis that respiratory tract epithelial cells have the potential to regulate T cell responses and play a role in tolerance to airborne antigens.
Figure 3. Effect of treatment with interferon (IFN)-γ on programmed death–1 ligand (PD-L1), PD-L2, B7-H3, and inducible costimulator ligand (ICOS-L) expression on NCI-H292, BEAS-2B, and A549 cells. Shown are the time courses of IFN-γ modulation of PD-L1, PD-L2, B7-H3, and ICOS-L expression on NCI-H292 (A), BEAS-2B (B), and A549 (C) cells. Cells cultured in medium (white squares) or RSV and IFN-γ (50 ng/mL; black triangles) were harvested at 4, 24, or 48 h and stained for PD-L1, PD-L2, B7-H3, or ICOS-L expression. Results are expressed as the fold induction of mean fluorescence intensity (MFI), compared with the MFI of cells treated with medium only. Data are means ± SEs of 4 experiments. In a separate experiment (D), A549 cells were treated with medium only (white bars) or with IFN-γ at different concentrations (1, 5, 50, or 100 ng/mL; hatched bars) and then were harvested at 24 h (48 h for B7-H3); examined by indirect immunofluorescence staining for PD-L1, PD-L2, B7-H3, or ICOS-L expression; and assessed by flow-cytometric analysis. Results are expressed as the fold induction of MFI, compared with the MFI of cells treated with medium only. Data are means ± SEs of 4 experiments. *P < .05; **P < .01; ***P < .001. NS, not significant.
It has been reported that treatment with cytokines such as TNF-α, IFN-γ, IL-4, and combinations thereof down-regulates ICOS-L expression on BEAS-2B cells and PBECs, compared with that on unstimulated epithelial cells [29], and that PD-L1 and ICOS-L expression on respiratory tract epithelial cells is increased after IFN-γ treatment [30]. In the present study, IL-4 treatment did not have any effect on any of the molecules studied, and IFN-γ treatment did not have any effect on ICOS-L expression but did increase PD-L1 expression on all 3 respiratory tract cell lines studied. In addition, we found that IFN-γ treatment also up-regulated PD-L2 expression on all 3 cell lines and down-regulated B7-H3 expression on RSV-infected bronchial and alveolar epithelial cells.

IFN-γ treatment up-regulated RSV-induced PD-L1 and PD-L2 expression—with higher PD-L1 than PD-L2 expression—on alveolar epithelial cells, suggesting a role in dampening/finishing an excessive type 1 immune response. We found that, on alveolar epithelial cells, the up-regulation of RSV-induced PD-L1 expression in the presence of IFN-γ was accompanied by the down-regulation of B7-H3 and ICOS-L expression. The proinflammatory activity of T cells might be regulated by IFN-γ via a negative feedback loop: activated Th1 cells, producing high levels of IFN-γ, might reduce their cytokine levels via IFN-γ–induced PD-L1 expression, as reported elsewhere [20, 30] and confirmed in the present study on respiratory tract epithelial cells. This negative regulatory feedback loop could be important for APC–T cell interactions in down-regulating or counterbalancing peripheral inflammatory activity [21]. We speculate that this might allow the lung epithelium to suppress the T cell antiviral immune responses.

IL-4 has been previously reported to increase PD-L2 expression on human macrophages [20]. In the present study, we found that the presence of excess IL-4 during RSV infection of alveolar epithelial cells, as occurs in atopic asthma, increased RSV-induced PD-L2 expression. IL-4 treatment also up-regulated RSV-induced B7-H3 expression and decreased RSV-induced ICOS-L expres-
sion on alveolar epithelial cells. This modulation may restrict the ability of respiratory tract epithelial cells to induce strong Th1 immune responses against RSV in an enriched type 2 cytokine milieu. These combined effects may facilitate the escape of RSV from surveillance by the host immune system and possibly cause chronic infection. We are planning future studies to address whether RSV-induced inhibitory molecule expression on human respiratory tract epithelial cells is functional and whether the blockage of the interactions between ligands and receptors augments antiviral immunity.

References