Interleukin-13 induces a hypersecretory ion transport phenotype in human bronchial epithelial cells

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Am J Physiol Lung Cell Mol Physiol 282: L226–L236, 2002. First published October 5, 2001; 10.1152/ajplung.00311.2001.—Interleukin (IL)-13 has been associated with asthma, allergic rhinitis, and chronic sinusitis, all conditions where an imbalance in epithelial fluid secretion and absorption could impact upon the disease. With the exception of submucosal glands, the bronchial epithelium can be modeled in vitro to display a differentiated mucociliary phenotype with the ion transport characteristics of the native tissue. To date, there is only one report of the effects of inflammatory stimuli on the ion transport function of the human airway epithelium (13). Galietta and colleagues (13) described the effects of the T-helper (Th) 1 cytokines interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) on the ion transport characteristics of human bronchial epithelial cells (HBECS) and demonstrated that TNF-α was without effect, although the basal amiloride-sensitive short-circuit current (Isc) was reduced by IFN-γ and agonist-stimulated anion-secretion was enhanced. Currently, there are no published reports of the effects of Th2 cytokines on the ion transport characteristics of the human airway epithelium. In this study, we report the effects of the Th2 cytokine interleukin (IL)-13 on the ion transport phenotype of the human bronchial epithelium. Increased IL-13 production is recognized in asthma (atopic and nonatopic), chronic sinusitis, and allergic rhinitis (16, 18, 19, 22, 31), all conditions in which alterations in the volume and composition of secretions and the epithelial lining fluid could impact the normal functioning of the tissue. The effects of IL-13 on the ion transport characteristics of other epithelia have been reported. IL-13 has been demonstrated to decrease transepithelial resistance (Rtp) of cultured T84 monolayers (39), but in contrast to the related Th2 cytokine IL-4 was without effect on agonist-stimulated anion secretion. In cultured rat glomerular visceral epithelial cells, both IL-4 and IL-13 decreased Rtp, an effect that was attributed to an increase in transcellular conductance (35). In this paper, we demonstrate that IL-13 and IL-4 are able to convert the human bronchial epithelium from its normal absorptive state to a secretory phenotype. This phenomenon may represent a potential mechanism by which...
the atopic airway can become hypersecretory and could highlight novel therapeutic approaches to treat airway diseases associated with imbalances of fluid secretion and absorption (32).

METHODS

Cell Culture

HBECs (Biowhittaker) were cultured using a modification of the method described by Gray and colleagues (15). Cells were seeded in plastic T-75 flasks and were grown in bronchial epithelial cell growth medium (BEGM; Biowhittaker) supplemented with bovine pituitary extract (52 μg/ml), hydrocortisone (0.5 μg/ml), human recombinant epidermal growth factor (0.5 ng/ml), epinephrine (0.5 μg/ml), transferrin (10 μg/ml), insulin (5 μg/ml), retinoic acid (0.1 μg/ml), triiodothyronine (6.5 μg/ml), gentamycin (50 μg/ml), and amphotericin B (50 μg/ml). Medium was changed every 48 h until cells were 90% confluent. Cells were then passaged and seeded (8.25 × 10⁴ cells/insert) on polycarbonate Snapwell inserts (Costar) in differentiation media containing 50% DMEM in BEGM with the same supplements as above but without amphotericin B or triiodothyronine and a final retinoic acid concentration of 50 nM (all-trans retinoic acid). Cells were maintained submersed for the first 7 days in culture, after which time they were exposed to an apical air interface for the remainder of the culture period. Cells were used between days 14 and 21 after establishment of the apical-air interface. At all stages of culture, cells were maintained at 37°C in 5% CO₂ in an air incubator. HBECs from three donors were used for these studies.

Iₑ Measurements

Snapwell inserts were mounted in Vertical Diffusion Chambers (Costar) and were bathed with continuously gassed Ringer solution (5% CO₂ in O₂; pH 7.4) maintained at 37°C (mean ± SE). Solutions were gassed with air. In studies to evaluate the basolateral membrane K⁺ conductance (Gₑ), the apical membrane was permeabilized with amphotericin B (10 μM; apical side only) with the apical solution containing potassium gluconate (120 mM) in place of NaCl and the basolateral solution containing sodium gluconate, again in the place of NaCl. Amphotericin B was added to the apical membrane 5–10 min after voltage clamping and was present throughout the experiment. In studies to evaluate the contribution of apical Gₑ and chloride conductance (G_Cl), the basolateral membrane was permeabilized with α-toxin (200 U/ml; basolateral side). For apical Gₑ studies, the basolateral side was bathed in a solution containing potassium gluconate (120 mM) in place of NaCl, and the apical solution contained sodium gluconate, again in the place of NaCl. In the apical G_Cl studies, the cells were initially bathed in equimolar normal Ringer solution, and after the addition of α-toxin the apical chloride concentration was reduced to 20 mM by performing serial dilutions with chloride-free Ringer (NaCl replaced by sodium gluconate). In all gluconate-containing solutions, the Ca²⁺ concentration was increased to 4 mM to compensate for the Ca²⁺-chelating property of gluconate (8).

Cytokine Treatment and Compound Additions

Initially, HBECs were treated basolaterally with the cytokines IL-13 (10 ng/ml) or IL-4 (10 ng/ml) for 48 h. Cytokine or vehicle-containing medium was refreshed at 24 h. At 48 h, the basal characteristics of the cells in addition to the amiloride-sensitive Iₑ (10 μM; apical side) were recorded. The subsequent responses to UTP (30 μM; apical side), ionomycin (1 μM; apical and basolateral), and forskolin (0.6 μM; apical and basolateral) were also assessed. In additional experiments, the sensitivity of the responses to bumetanide (60 μM; basolateral) and DIDS (300 μM; apical) were examined. The effect of UTP on control and IL-13-treated HBECs was also assessed in the absence of amiloride.

Effects of IL-13 on UTP-Stimulated Intracellular Ca²⁺

HBECs were seeded on clear-bottomed, black-walled 96-well tissue culture-treated plates (Costar) at 20,000 cells/well in differentiation media with or without IL-13 (10 ng/ml). At 48 h after seeding, cells were loaded with fluo 4-AM (0.7 μM in DMSO + 20% pluronic acid, Molecular Probes) in loading buffer containing differentiation media, HEPES (20 mM), and probenecid (2.5 mM) at 37°C (5% CO₂) for 60 min. The final DMSO concentration did not exceed 0.1% vol/vol. The cells were then washed three times by rinsing with wash buffer containing Hanks’ balanced salt solution (with Ca²⁺, Mg²⁺ without phenol red), HEPES (20 mM), and probenecid (2.5 mM), and the final volume was adjusted to 100 μl/well (Labsystems Cellwash Microplate Washer). Fluorescence intensity was then continuously measured before and after the addition of UTP (final concentration 0.1–100 μM) using FLIPR (FLuorescence Imaging Plate Reader; Molecular Devices) with excitation and emission wavelengths at 488 and 535 nm, respectively.

Histology

HBECs were treated with vehicle or IL-13 (10 ng/ml; 48 h) as described above and were then fixed in 10% neutral-buffered formalin (pH 7.4; 24 h). Inserts were then processed and embedded in wax. Sections (3 μm) were mounted on glass slides and dried overnight before staining (Alcian blue and hematoxylin). The numbers of goblet cells on the epithelium were counted and expressed as the percentage of the total number of epithelial cells on the apical surface. A total of four sections was used from each insert, and the entire length of the insert was used for scoring. Each group consisted of six individual inserts.

Expression of Results and Statistical Analysis

Results are expressed as absolute changes in Iₑ (mean ± SE). Measurements were taken either as peak changes or once responses had plateaued and were stable. Control inserts were run alongside all experiments for paired comparisons to be made because of the potential day-to-day and interbatch variability of the Iₑ. Student’s t-test was used to compare between groups, with statistical significance assumed at P < 0.05. For the FLIPR studies, data are expressed as a percentage of the maximum response to UTP (mean ± SE).
IL-13-INDUCED HYPERSECRETORY PHENOTYPE

Fig. 1. Sample current traces showing the effects of a 48-h incubation with vehicle (A), interleukin (IL)-4 (10 ng/ml; B), and IL-13 (10 ng/ml; C) on the basal and stimulated short-circuit current ($I_{sc}$) characteristics of human bronchial epithelial cells (HBECs). Vertical deflections represent the $I_{sc}$ response to a ±2-mV pulse. The following concentrations were used: amiloride, 10 μM (apical); UTP, 30 μM (apical); forskolin (FK), 0.6 μM (apical + basolateral).

Reagents

HBECs obtained from postmortem specimens were purchased from Biowhittaker, as were all media. All other cell culture reagents were purchased from Life Technologies. Cytokines were purchased from (PeproTech). All other reagents were purchased from Sigma, unless stated otherwise.

RESULTS

The culture methods employed in these studies produced a multilayered bronchial epithelial tissue that had differentiated to the extent that ciliated and goblet cells were identifiable. Goblet cells typically accounted for 25–30% of the total number of cells at the apical surface (see Histology). All of the control cells used in these studies displayed an amiloride-sensitive $I_{sc}$, although there was inevitable inter- and intradonor variability. Paired controls were used throughout.

IL-4 and IL-13 Inhibit Basal and Amiloride-Sensitive $I_{sc}$ but Enhance the Responses to UTP and Forskolin

Basal $I_{sc}$. Initial studies investigated the effects of IL-4 and IL-13 on the basal and stimulated ion transport properties of HBECs (Fig. 1). Control cells displayed a basal $I_{sc}$ of 34.7 ± 1.4 μA/cm² and $R_T$ of 846 ± 62 Ω·cm² (n = 6). Amiloride inhibited 72.8 ± 3.2% of the basal current (n = 6). In contrast, cells that had been treated with IL-4 (10 ng/ml) or IL-13 (10 ng/ml) displayed significantly reduced basal currents of 5.7 ± 0.3 μA/cm² ($P < 10^{-6}$; n = 6) and 5.3 ± 0.7 μA/cm² ($P < 10^{-6}$; n = 6), respectively. IL-4- and IL-13-treated cells also showed an increased $R_T$ of 1,391 ± 162 Ω·cm² ($P < 0.02$) and 1,580 ± 112 Ω·cm² ($P < 10^{-4}$), respectively. Furthermore, <2% of the basal current was amiloride-sensitive in both the IL-4- and IL-13-treated cells ($P < 10^{-2}$; n = 6).

$UTP$-stimulated $I_{sc}$. The subsequent addition of UTP (30 μM; apical) in the presence of amiloride induced a biphasic $^1$ increase in $I_{sc}$ in both control and cytokine-treated cells (Fig. 1). In control cells, the current peaked at an increase of 7.1 ± 0.3 μA/cm² (n = 6). In IL-4- and IL-13-treated cells, the $I_{sc}$ peaked at an increase of 50.9 ± 0.9 μA/cm² ($P < 10^{-12}$; n = 6) and 44.3 ± 2.2 μA/cm² ($P < 10^{-8}$; n = 6), respectively. Changes in $R_T$ were also associated with these $I_{sc}$ changes. In control cells, mean $R_T$ decreased from 1,038 ± 31 to 824 ± 64 Ω·cm² (n = 6) upon the addition of UTP. In IL-4- and IL-13-treated cells, $R_T$ was likewise reduced after UTP stimulation from 1,650 ± 267 to 487 ± 17 Ω·cm² (n = 6) and 1,655 ± 192 to 541 ± 18 Ω·cm² (n = 6), respectively. It should, however, be noted that these values could only be calculated after the peak response had reached a steady plateau and do not necessarily represent the true value of $R_T$ at the time of the peak increase in $I_{sc}$.

Forskolin-stimulated $I_{sc}$. After the resolution of the UTP response, the baseline $I_{sc}$ remained elevated over the pre-UTP level in control (139 ± 6%; $P < 0.02$; n = 6).

$^1$For clarity we have quantified only the peak increase in $I_{sc}$ throughout.
6) and IL-4 (199 ± 15%; P < 10^-6; n = 6) and IL-13 (199 ± 24; P < 10^-4; n = 6)-treated cells. The subsequent addition of forskolin (0.6 μM; apical and basolateral) induced a peak increase in Isc of 9.7 ± 1.1 μA/cm^2 (n = 6) in control cells that was significantly elevated in the IL-4- and IL-13-treated cells to an increase of 15.0 ± 0.5 μA/cm^2 (P = 0.001; n = 6) and 15.4 ± 1.2 μA/cm^2 (P = 0.003; n = 6), respectively.

**IL-13-Induced Effects on the HBEC Ion Transport Phenotype Are Apparent at 6 h**

Initially, HBECs were treated with IL-13 (10 ng/ml; basolateral) for 24, 48, and 72 h to determine whether the phenomenology observed above was dependent on the duration of treatment. In this study, control cells (receiving fresh media at 0, 24, and 48 h) displayed a basal Isc of 20.2 ± 0.8 μA/cm^2 (n = 6). In the IL-13-treated cells, the basal Isc was reduced to 9.4 ± 1.1, 5.8 ± 0.6, and 8.6 ± 0.5 μA/cm^2 in the 24-, 48-, and 72-h treatment groups, respectively. The amiloride-sensitive current was also completely inhibited in all IL-13-treated groups (P < 10^-4; n = 4–6; Fig. 2). At all of the time points studied, IL-13 significantly increased the UTP-stimulated increase in Isc. The peak increase in UTP-stimulated Isc was increased from 11.8 ± 0.6 μA/cm^2 in the control cells to 71.5 ± 4.1, 99.4 ± 6, and 107.6 ± 2.4 μA/cm^2 with 24, 48, and 72 h of IL-13 treatment, respectively. There was a significant increase in the response to UTP between 24- and 48-h treatments (P < 0.01) but not between 48 and 72 h. After the resolution of the UTP response, the increase in Isc induced by forskolin was also significantly elevated from 14.3 ± 0.5 μA/cm^2 in the control cells to 23.6 ± 1.7 (P < 0.02), 37.7 ± 2.5 (P < 0.001), and 45.3 ± 2.7 (P < 10^-4) μA/cm^2 in the 24-, 48-, and 72-h treatment groups, respectively. Likewise, there was a significant increase in the forskolin-stimulated Isc response between 24 and 48 h (P < 0.004) but not between 48 and 72 h of IL-13 treatment. Because of the complete attenuation of the amiloride-sensitive Isc by 24 h, a subsequent study examined the effects of IL-13 treatment of HBECs for 2 and 6 h (with a 48-h treatment as a positive control). In this study, there was no effect of IL-13 until 6 h. At this time, the basal and amiloride-sensitive currents were reduced from 27.5 ± 1.6 and 15.0 ± 1.3 μA/cm^2, respectively, in control cells and to 14.3 ± 1.7 (P < 10^-3; n = 5) and 5.0 ± 1.6 (P < 10^-3; n = 5) μA/cm^2 in the IL-13-treated group. The peak response to UTP was also enhanced at 6 h from 9.3 ± 0.9 μA/cm^2 in the control cells to 15.6 ± 0.9 μA/cm^2 in the IL-13 group (P < 10^-3; n = 5). The subsequent response to forskolin was also enhanced from 15.1 ± 1.4 to 22.6 ± 2.4 μA/cm^2 (P = 0.03; n = 5) after 6 h of IL-13 treatment. The 48-h treatment was chosen for all subsequent studies.

**Inhibitory Isc Response to UTP is Lost in IL-13-Treated HBECs**

As previously observed, HBECs that had been treated with IL-13 (10 ng/ml; 48 h) had a significantly reduced basal Isc of 10.4 ± 0.3 μA/cm^2 compared with 14.1 ± 0.3 μA/cm^2 in the control cells (P < 10^-5; n = 6). The addition of UTP (30 μM apical) to the control cells induced a transient increase in Isc of 6.2 ± 0.8 μA/cm^2 followed by a sustained inhibitory phase that reached a steady baseline at 3.9 ± 0.6 μA/cm^2 below the starting, pre-UTP current (n = 6; Fig. 3). In contrast, the IL-13-treated cells responded to UTP with a peak increase in Isc of 95.4 ± 3.1 μA/cm^2 (P < 10^-10; n = 6) that remained elevated above the baseline Isc for the duration of the experiment.

**IL-13 Enhances Forskolin-Stimulated Isc Under Basal Conditions**

In a subsequent study, HBECs that had been treated with IL-13 (10 ng/ml; 48 h) again showed a reduced basal Isc of 6.9 ± 0.8 μA/cm^2 compared with 15.9 ± 0.5 μA/cm^2 in control cells (P < 10^-4; n = 9). The addition of forskolin under these basal conditions induced a sustained increase in Isc of 12.6 ± 0.7 μA/cm^2 in control cells that was significantly enhanced in the IL-13-treated cells to an increase of 24.4 ± 1.3 μA/cm^2 (P < 10^-6; n = 9).

**IL-13 Treatment Does Not Affect Goblet Cell Density**

In control cells, goblet cells accounted for 26.3 ± 3.6% of the cells at the apical surface of the epithelium (n = 6). In paired cells treated for 48 h with IL-13 (10 ng/ml), 31.0 ± 3.3% of the cells at the apical surface of the epithelium were goblet cells (P = 0.36, n = 6).

**Sensitivity of the UTP-Stimulated Increase in Isc to Bumetanide and DIDS**

We next investigated the nature of the increased responsiveness to UTP in IL-13-pretreated cells using bumetanide, a blocker of the basolateral Na^+–K^+–2Cl^- cotransporter, and DIDS, a nonselective blocker of an-
ion channels that is without effect on the cystic fibrosis transmembrane conductance regulator (CFTR; see Ref. 33). In the control cells, UTP stimulated a peak increase in $I_{sc}$ of $8.3 \pm 0.5 \mu A/cm^2$ ($n = 6$). The subsequent addition of bumetanide (60 μM; basolateral) reduced the current by $11.3 \pm 0.8 \mu A/cm^2$ (Fig. 4A). In the IL-13-treated cells, UTP stimulated an increase in $I_{sc}$ of $37.1 \pm 1.9 \mu A/cm^2$ when measured at the plateau phase of the response (Fig. 4B). The addition of bumetanide reduced the current by $33.8 \pm 2.5 \mu A/cm^2$ (Fig. 4B).

For the studies with DIDS, ionomycin was used in place of UTP, as DIDS has been demonstrated to block P2Y2 receptors (33). In IL-13-treated cells, ionomycin (1 μM; apical and basolateral) induced a biphasic increase in $I_{sc}$ that peaked at $50.7 \pm 6.9 \mu A/cm^2$, a significantly larger response than observed in the control cells of $4.9 \pm 0.5 \mu A/cm^2$ ($P < 10^{-4}$; $n = 6$; Fig. 5, A and B). The addition of DIDS before ionomycin did not affect the peak increase in $I_{sc}$ in the control cells (4.1 ± 0.6 μA/cm²; $P = 0.35$; $n = 6$; Fig. 5C). In the IL-13-treated cells, DIDS attenuated the ionomycin-stimulated peak increase in $I_{sc}$ from $50.7 \pm 6.9$ to $21.4 \pm 2.3 \mu A/cm^2$ ($P = 0.002$; $n = 6$; Fig. 5D). The forskolin-stimulated $I_{sc}$ responses were unaffected by DIDS in both control (23.7 ± 1.1 vs. 22.3 ± 1.0 μA/cm²) and IL-13-treated cells (57.8 ± 2.9 vs. 50.8 ± 2.2 μA/cm²).

Under low-chloride and bicarbonate-free conditions, the UTP-stimulated increase in $I_{sc}$ was attenuated in both control cells and cells that had been pretreated with IL-13. In control cells, the peak UTP responses were reduced from $10.6 \pm 0.2$ (normal Ringer) to $2.8 \pm 0.3$ (low chloride, bicarbonate free; $n = 3$) μA/cm². Likewise, in IL-13-treated cells, the peak responses were reduced from $63.9 \pm 1.5$ (normal Ringer) to $30.5 \pm 1.2$ (low chloride, bicarbonate free; $n = 3$; Fig. 6) μA/cm². Under the low-chloride bicarbonate-free conditions, the recovery of the $I_{sc}$ response toward baseline was more rapid than in the paired control (Fig. 6).

**IL-13-Induced Effects on Apical $G_{Cl}$**

To determine whether IL-13 increased the UTP-stimulated apical $G_{Cl}$ of HBECs, cells were treated in Ussing chambers with amiloride and then α-toxin (200 U/ml; basolateral). In these cells, the basal and amiloride-sensitive currents were reduced from $20.4 \pm 0.6$ and $9.8 \pm 0.8 \mu A/cm^2$ ($n = 11$) in control cells and to $12.2 \pm 1.0$ ($P < 10^{-6}$; $n = 9$) and $2.0 \pm 0.4$ ($P < 10^{-6}$; $n = 9$) μA/cm², respectively, in the IL-13 treated cells (Fig. 7). The addition of α-toxin reduced the $I_{sc}$ values for control and IL-13-treated cells by $14.5 \pm 0.8$ and $13.4 \pm 1.3 \mu A/cm^2$, respectively. The establishment of a basolateral-to-apical chloride gradient by diluting the...
apical chloride concentration to 20 mM induced an increase in $I_{sc}$ of $16.6 \pm 3.6$ and $13.9 \pm 3.2$ $\mu$A/cm$^2$ in control and IL-13-treated cells, respectively. Stimulation of the cells with UTP induced an increase in $I_{sc}$ in both groups, as previously observed. In the control cells, $I_{sc}$ peaked at an increase of $31.4 \pm 3.0$ $\mu$A/cm$^2$ compared with an increase of $99.0 \pm 7.9$ $\mu$A/cm$^2$ ($P < 10^{-8}; n = 9–11$) in the IL-13-treated cells. Data are summarized in Fig. 7C.

**IL-13-Induced Effects on Basal and Stimulated $K^+$ Currents**

The potential contribution of basolateral and apical $K^+$ currents to the enhanced UTP response after IL-13 treatment was studied by selectively permeabilizing either membrane while under an established $K^+$ gradient.

**Basolateral $G_K$.** Under an applied apical-to-basolateral $K^+$ gradient, the addition of amiloride reduced the

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**Fig. 5.** Sample current traces showing the effects of DIDS on the ionomycin-stimulated increase in $I_{sc}$ in HBECs. DIDS (300 $\mu$M, apical) attenuated the ionomycin-stimulated increase in $I_{sc}$ in IL-13-pretreated cells (10 ng/ml, 48 h; B and D). DIDS was without effect on the subsequent forskolin-stimulated increase in $I_{sc}$ (C). All experiments were performed in the presence of amiloride. Vertical deflections represent the $I_{sc}$ response to a $ \pm 2$-mV pulse. ION, ionomycin (1 $\mu$M, apical + basolateral).

**Fig. 6.** Sample current traces showing the effect of removal of chloride and bicarbonate from the Ringer solution. In low-chloride, bicarbonate-free solution (A), the UTP (30 $\mu$M, apical)-stimulated $I_{sc}$ response was attenuated when compared with the control cells in normal Ringer (B). All cells had been pretreated with IL-13 (10 ng/ml, 48 h), and experiments were performed in the presence of amiloride. Vertical deflections represent the $I_{sc}$ response to a $ \pm 2$-mV pulse.
basal $I_{sc}$ by 3.5 ± 0.4 (n = 6) and 1.0 ± 0.2 (n = 6) μA/cm² in the control and IL-13-treated cells, respectively, indicating that the IL-13 treatment had affected the ion transport phenotype, as previously seen. The addition of amphotericin B (10 μM) to the apical surface induced a slow and sustained increase in $I_{sc}$ (Fig. 8) that has previously been demonstrated to be due to basolateral $G_K$. There was no difference in $G_K$ between control and IL-13-treated cells (control increased by 58.5 ± 6.1 μA/cm² and IL-13-treated increased by 66.2 ± 6.9 μA/cm², $P = 0.42$). The subsequent addition of UTP induced a transient increase in $I_{sc}$ in both control and IL-13-treated cells of 102.7 ± 5.9 and 95.5 ± 4.9 μA/cm², respectively ($P = 0.39$, n = 6), that was followed by a reduction in the basal $G_K$, as has been previously described in HBECs (10).

Apical $G_K$. Under an applied basolateral-to-apical K⁺ gradient, the addition of α-toxin (200 U/ml) to the basolateral membrane induced a biphasic reduction in $I_{sc}$ in control cells that reached a plateau after ~30 min of −46.5 ± 7.3 μA/cm² (Fig. 9A). In contrast, the current decrease induced by α-toxin in the IL-13-treated cells was significantly lower at −11.2 ± 2.9 μA/cm² ($P < 0.002$, n = 6; Fig. 9B). The subsequent addition of UTP induced a further decrease in $I_{sc}$ of −44.3 ± 5.0 μA/cm² in the control cells and −49.5 ± 4.8 μA/cm² in the IL-13-treated cells ($P = 0.47$, n = 6).

In the control cells, the $I_{sc}$ reached a steady baseline at −1.3 ± 1.6 μA/cm² compared with −9.1 ± 2.1 μA/cm² in the IL-13-treated cells ($P < 0.02$, n = 6).

**IL-13 Does Not Affect Agonist-Induced Increases in Intracellular Ca²⁺ Concentration**

HBECs cultured on plastic for 48 h either in the presence or absence of IL-13 (10 ng/ml) responded to UTP in a concentration-dependent manner with an increase in intracellular Ca²⁺ concentration. There were no differences in either the sensitivity or magnitude of the response induced by IL-13 (Fig. 10). IL-13 was likewise without effect on the ionomycin-induced increase in intracellular Ca²⁺ concentration (data not shown).

**DISCUSSION**

IL-13 and IL-4 are key mediators of Th2-type inflammatory responses in conditions such as asthma, allergic rhinitis, and chronic sinusitis (16, 18, 19, 22, 31). Elevated levels of these cytokines have been demonstrated in disease, and transgenic mice producing increased levels of these proteins present with an airway hypersecretory phenotype and goblet cell metaplasia (33, 38). To date, there are no published studies examining the effects of Th2 cytokines on the ion transport...
because it is the apical epithelial Na\(^+\) channel (ENaC) that is the rate-limiting step for amiloride-sensitive Na\(^+\) absorption in this tissue, it is likely that it is the result of a loss of this apical Na\(^+\) conductance. A loss of ENaC function could be because of a direct reduction in expression of one or more of the ENaC genes or alternatively to an increase in the expression of a negative regulator of ENaC function, such as CFTR (24). An alternative mechanism behind the loss of the amiloride-sensitive \(I_{sc}\) in this study could be the inhibition of the apical \(G_K\) identified in the \(\alpha\)-toxin permeabilization experiments (Fig. 9). A higher apical \(G_K\) in control cells would tend to hyperpolarize the apical membrane and thereby decrease the driving force for Na\(^+\) entry. Conversely, an inhibition of the apical \(G_K\) by IL-13 would tend to depolarize the apical membrane and thereby decrease the driving force for Na\(^+\) entry and inhibit Na\(^+\) absorption. This reduction in apical \(G_K\) may also contribute to the IL-13-induced increase in \(R_T\) that was observed. However, an IL-13-induced effect on the ba-

![Fig. 8. Sample current traces showing the lack of effect of IL-13 on basolateral K\(^+\) conductance (\(G_K\)) in HBECs. Under an established apical-to-basolateral K\(^+\) gradient, permeabilization of the apical membrane with amphotericin B (10 \(\mu\)M) induced an increase in \(I_{sc}\) that was the result of \(G_K\). This basal \(G_K\) was not different between control (A) and IL-13-pretreated (B) cells. The change in \(G_K\) stimulated by UTP was not different between control and IL-13-treated cells. Vertical deflections represent the \(I_{sc}\) response to a \(\pm\)2-mV pulse.](#)

![Fig. 9. Sample current traces showing the effects of IL-13 on apical \(G_K\) in HBECs. Under an established basolateral-to-apical K\(^+\) gradient, the addition of \(\alpha\)-toxin (200 \(U/ml\)) to the basolateral membrane of control cells (A) induced a slow decrease in \(I_{sc}\) that was the result of the permeability of the apical membrane to K\(^+\). In contrast, \(\alpha\)-toxin induced only a small decrease in \(I_{sc}\) in the IL-13-pretreated cells (B). There were no differences in the magnitude of the subsequent response to UTP. Vertical deflections represent the \(I_{sc}\) response to a \(\pm\)2-mV pulse.](#)
solateral or paracellular resistances cannot be ruled out, although the former is unlikely, as no effect of IL-13 on basolateral $G_K$ was observed (Fig. 8; see below). Few studies have examined the effects of inflammatory mediators on the amiloride-sensitive current in the airway. It has been demonstrated that TNF-α and growth factors such as keratinocyte growth factor can increase amiloride-sensitive currents in airway epithelial cells both in vitro and in vivo (3, 11, 36). Conversely, studies have also demonstrated that inflammatory stimuli can decrease amiloride-sensitive currents in both culture and ex vivo tissue samples (20, 23). The mechanism(s) underlying the IL-4- and IL-13-mediated inhibition of the amiloride-sensitive $I_{sc}$ in this study will require further investigation.

The most striking effects of IL-4 and IL-13 in this study were the enhancement of the UTP and ionomycin-stimulated increases in $I_{sc}$. UTP was chosen as a Ca$^{2+}$-mobilizing agonist for these studies, since the effects of nucleotide triphosphates have been widely characterized in the human airway epithelium. Ionomycin was used to demonstrate that the IL-13-induced enhanced UTP response was a receptor-independent effect. Furthermore, we have demonstrated that IL-13 does not affect the mobilization of intracellular Ca$^{2+}$ induced by UTP. However, it should be considered that the Ca$^{2+}$- studies were performed on HBECS cultured on plastic and could potentially behave differently to the polarized epithelia. Ionomycin was also used in the studies involving DIDS, since this chloride channel-blocking compound has been demonstrated to antagonize $P_2$ receptors (33). The initial experiments (Figs. 1 and 2) demonstrated that, in the presence of amiloride, UTP induced an increase in $I_{sc}$ that was significantly larger after IL-13 treatment. It was therefore necessary to determine whether a similar effect was apparent in a more physiologically relevant, amiloride-free situation. In the absence of amiloride, UTP induced a transient increase in $I_{sc}$ in control cells that was followed by a sustained inhibitory phase, consistent with the observations of Devor and Pilewski (10; Fig. 3A). In contrast, cells that had been treated with IL-13 developed an enhanced UTP-stimulated increase in $I_{sc}$ similar to that observed in the presence of amiloride (Fig. 3B). This is of relevance, since Ca$^{2+}$-mobilizing agonists appear to only inhibit Na$^+$ absorption in the healthy airway, but these same agonists can clearly cause an anion secretory response in inflamed airways. All further characterization of the UTP-induced secretory $I_{sc}$ was performed in the presence of amiloride to remove the potential complication of the effect on the Na$^+$ current.

The mechanisms responsible for $I_{sc}$ changes induced by Ca$^{2+}$-mobilizing stimuli in the airway epithelium are not fully understood but are likely to involve the concerted effects of apical CFTR and an as-yet-unidentified apical Ca$^{2+}$-activated $G_{Cl}$ combined with the apical and basolateral $G_K$ (9, 30, 37). A recent study reported by Paradiso and colleagues (30) demonstrated that UTP was able to activate both a Ca$^{2+}$-activated $G_{Cl}$ and CFTR, the latter through a protein kinase C-mediated effect. The study by Paradiso et al. (30) also demonstrated that the transient nature of the $I_{sc}$ changes induced by UTP was mirrored by the transient increase in intracellular Ca$^{2+}$ concentration and that manipulations designed to attenuate rises in intracellular Ca$^{2+}$ concentration also reduced the $I_{sc}$ changes. UTP has also been reported to stimulate two independent $G_{Cl}$ using the perforated-patch technique with HBECS (37). It is apparent that an effect of IL-4 or IL-13 on either the apical $G_{Cl}$ and/or basolateral $G_K$ could manifest as an increase in an anion secretory response. An immune-mediated increase in a Ca$^{2+}$-activated $G_K$ is not without precedent, as an anti-CD3 antibody has been demonstrated to increase the expression of hIKCa1 in human T cells (14). However, in this study, the apical permeabilization experiments (Fig. 8) showed that IL-13 had no effect on the basolateral $G_K$ under basal or UTP-stimulated conditions. Galietta and colleagues (13) recently reported that IFN-γ treatment enhanced the secretory response to Ca$^{2+}$-mobilizing agonists in their HBEC model and that the response was independent of the basolateral membrane. Devor et al. (9) also observed an apical UTP-stimulated secretory $G_K$ in their HBEC model that could also influence the net current observed in response to UTP. A secretory K$^+$ current could mask the magnitude of any anion secretory current or alternatively enhance an anion secretory response, since an increase in apical $G_K$ would be predicted to hyperpolarize the cell and thereby increase the driving force for anion secretion. The basolateral permeabilization study (Fig. 9) indicated that UTP stimulated an apical secretory K$^+$ current, as previously described (9). The peak increase in this current was unaffected by IL-13 pretreatment (Fig. 9). These observations therefore point to an IL-13- and/or IL-4-induced increase in an apical anion secretory conductance that was further supported by the bumetanide sensitivity and anion-dependent nature of the current. The DIDS sensitivity of the ionomycin-stimulated response (~60% inhibition
of the $I_{sc}$ response at 300 μM) further indicated that a significant proportion of the current was mediated through a conductance other than CFTR. It is also unlikely that an increase in CFTR expression would account for the increased UTP or ionomycin-stimulated currents as the forskolin response was increased by approximately two- to threefold while the UTP/ionomycin responses were increased by more than sixfold. Finally, the observation of an enhanced UTP-stimulated increase in $I_{sc}$ in HBECs under a chloride gradient (basolateral to apical) with the basolateral membrane permeabilized was conclusive evidence of an IL-13-induced functional Ca$^{2+}$-activated anion conductance in the apical membrane.

The only reports of Th2 cytokine-mediated effects on epithelial ion transport function have used T84 cells and glomerular visceral epithelial cells. In the T84 study, both IL-4 and IL-13 attenuated $R_T$, although only IL-4 affected chloride secretion through an inhibition of CFTR expression (39). In the glomerular visceral epithelial cells, both IL-4 and IL-13 increased basal $I_{sc}$; however, the ionic basis and mechanisms were not investigated (35). The molecular identity of the Ca$^{2+}$-activated $G_{Cl}$ in the airway epithelium are, however, unknown. Evidence is emerging that a family of putative Ca$^{2+}$-activated chloride channels (12) that include the murine gene gob-5 (mCLCA3) may play a role in epithelial inflammation; gob-5 has recently been demonstrated to be upregulated and to play a key role in the development of an asthma phenotype in vivo in the airways of allergen-challenged mice (28). It remains to be determined whether the IL-13-induced Ca$^{2+}$-activated $G_{Cl}$ reported here is indeed a member of this family. The effects of inflammatory stimuli on the expression of CFTR in epithelia have been studied more widely. Evidence exists for both up- and down-regulation of CFTR by inflammatory stimuli in various epithelia (2, 5, 6, 13, 27). In HBECs, IFN-γ decreased CFTR expression (13). In Calu-3 cells, IL-1β has been demonstrated to increase CFTR expression through an nuclear factor-κB-mediated pathway (5), whereas in the gut epithelial cell lines T84 and HT-29 CFTR expression can be differentially regulated by IFN-γ and IL-1β (2, 6).

These data all lead to the conclusion that, during both Th1 and Th2 inflammatory responses in the airway, the bronchial epithelium can convert from an absorbing to a secretory phenotype. The purpose of this phenotype shift can only be speculated upon at present but may represent a “flushing” response to rinse particulate and secreted mucus out of the airway lumen to both prevent congestion and to remove the inflammatory stimuli. Cystic fibrosis also underlines the importance of the balance between fluid and secreted mucus in the airway, and it may be that the epithelium becomes secretory to balance the increase in mucus secretion that is evident during these inflammatory events. Furthermore, in pseudohypoaldosteronism type II, ENaC is dysfunctional, and patients have a fluid hypersecretory phenotype in the airways that is evident as rhinitis (21). What is surprising is that, in these patients, the rate of mucociliary clearance is upregulated by up to fivefold, and it may be that the bronchial epithelium converts to a hypersecretory phenotype during inflammatory events to elicit a pseudohypoaldosteronism type II clearance response. These observations may have consequences for both the treatment of hypersecretory diseases of the lung and potentially cystic fibrosis, where an enhanced anion secretion response that is independent of CFTR could serve to address the imbalance of airway fluid transport.

REFERENCES


