Cooperative Effects of Th2 Cytokines and Allergen on Normal and Asthmatic Bronchial Epithelial Cells

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In sensitized individuals, exposure to allergens such as *Dermatophagoides pteronyssinus* (Der p) causes Th2 polarization and release of cytokines, including IL-4 and IL-13. Because Der p extracts also have direct effects on epithelial cells, we hypothesized that allergen augments the effects of Th2 cytokines by promoting mediator release from the bronchial epithelium in allergic asthma. To test our hypothesis, primary bronchial epithelial cultures were grown from bronchial brushings of normal and atopic asthmatic subjects. RT-PCR showed that each culture expressed IL-4Rα, common γ-chain, and IL-13Rα2, as well as IL-13Rα1, which negatively regulates IL-13 signaling; FACS analysis confirmed IL-13Rα2 protein expression. Exposure of epithelial cultures to either Der p extracts, TNF-α, IL-4, or IL-13 enhanced GM-CSF and IL-8 release, and this was partially suppressible by corticosteroids. Simultaneous exposure of the epithelial cultures to IL-4 or IL-13 together with Der p resulted in a further increase in cytokine release, which was at least additive. Release of TGF-α was also increased by TNF-α and combinations of IL-4, IL-13, and Der p; however, this stimulation was only significant in the asthma-derived cultures. These data suggest that, in an allergic environment, Th2 cytokines and allergen have the potential to sustain airway inflammation through a cooperative effect on cytokine release by the bronchial epithelium. Our novel finding that IL-4, IL-13, and allergen enhance release of TGF-α, a ligand for the epidermal growth factor receptor that stimulates fibroblast proliferation and goblet cell differentiation, provides a potential link between allergen exposure, Th2 cytokines, and airway remodelling in asthma. The Journal of Immunology, 2002, 169:407–414.
bronchial epithelium (18, 19), but expression of IL-13Rα2 has not been investigated. IL-4 and IL-13 both signal via the transcription factor STAT-6 (14, 20), whose expression is prominent in the bronchial epithelium and is further increased in severe asthma (21). Genetic variation in several components of the IL-4 and IL-13 signal transduction pathway has been implicated in asthma susceptibility or severity (13, 22). For examples, the IL-4Ra gene on chromosome 16p12.1 has eight polymorphisms in the coding region leading to amino acid changes (23, 24). These include an extracellular variant, I50V, that up-regulates receptor responses to IL-4, leading to increased STAT-6 activation and IgE synthesis (25, 26). Q576R and a single nucleotide polymorphism in the intracellular domain located in the STAT-6 binding region enhance signaling and are strongly associated with asthma severity (27).

In view of the ability of allergen to affect epithelial function directly by protease-dependent mechanisms and indirectly through immune cell activation and secretion of Th2 cytokines, the purpose of this study was to analyze the single and combined effects of immune cell activation and secretion of Th2 cytokines, the purpose of this study was to analyze the single and combined effects of IL-4, IL-13, and allergen extracts on the secretory properties of bronchial epithelial cells. As cell responses may be influenced by the type of receptors expressed, we also analyzed the IL-4R and IL-13R subunits present on bronchial epithelial cells and related their expression to functional outcome.

### Materials and Methods

#### Subjects

For bronchial brushing, nonatopic, nonasthmatic control subjects (n = 19) and asthmatic subjects (n = 19) were characterized according to symptoms, pulmonary function, and medication. Assessment of asthma severity was in accordance with the Global Initiative for Asthma guidelines on the diagnosis and management of asthma (28). The mild asthmatics were receiving inhaled β2-agonists (salbutamol) only, while the moderate-severe group was maintained on inhaled corticosteroids, plus or minus long acting β2-agonists (Table I). All subjects were nonsmokers and were free from respiratory tract infections for a minimum of 4 wk before inclusion to the study. The moderately severe asthmatic subjects treated with inhaled corticosteroids withheld this medication for a minimum of 1 wk before bronchoscopy. Written informed consent was obtained from all volunteers before participation, and ethical approval was obtained from the Joint Ethics Committee of Southampton University Hospital Trust.

All subjects were tested for atopy using a panel of common allergens, and serum IgE levels were measured by standard ELISA. Airway hyperresponsiveness was assessed by histamine inhalation challenge, and expressed as PC_{20} (the cumulative dose of histamine required to produce a fall in forced expiratory volume in 1 s by 20% from baseline).

#### Fiberoptic bronchoscopy

Bronchial brushings were obtained by bronchoscope (Olympus FB-20D, Tokyo, Japan) in accordance with standard published guidelines (29). Bronchial epithelial cells were obtained using a standard sterile single-sheathed nylon cytology brush (Olympus BC 408 EPITHELIAL CELL RESPONSES TO ALLERGEN AND Th2 CYTOKINES

#### Table I. Clinical characteristics of atopic asthmatic subjects and healthy control subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Atopy</th>
<th>Asthma Severity</th>
<th>FEV1, a (% Pred)</th>
<th>PC_{20}</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>26</td>
<td>M</td>
<td>Yes</td>
<td>Mild</td>
<td>3.78</td>
<td>89</td>
</tr>
<tr>
<td>A2</td>
<td>29</td>
<td>F</td>
<td>Yes</td>
<td>Mild</td>
<td>3.07</td>
<td>91</td>
</tr>
<tr>
<td>A3</td>
<td>19</td>
<td>M</td>
<td>Yes</td>
<td>Mild</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>20</td>
<td>M</td>
<td>Yes</td>
<td>Mild</td>
<td>2.83</td>
<td>68</td>
</tr>
<tr>
<td>A5</td>
<td>23</td>
<td>M</td>
<td>Yes</td>
<td>Mild</td>
<td>5.15</td>
<td>100</td>
</tr>
<tr>
<td>A6</td>
<td>19</td>
<td>M</td>
<td>Yes</td>
<td>Mild</td>
<td>3.95</td>
<td>99.7</td>
</tr>
<tr>
<td>A7</td>
<td>27</td>
<td>M</td>
<td>Yes</td>
<td>Mild</td>
<td>4.1</td>
<td>103</td>
</tr>
<tr>
<td>A8</td>
<td>19</td>
<td>F</td>
<td>Yes</td>
<td>Mild</td>
<td>3.05</td>
<td>94</td>
</tr>
<tr>
<td>A9</td>
<td>20</td>
<td>F</td>
<td>Yes</td>
<td>Mild</td>
<td>2.9</td>
<td>83</td>
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<tr>
<td>A10</td>
<td>25</td>
<td>M</td>
<td>Yes</td>
<td>Moderate-severe</td>
<td>3.06</td>
<td>69</td>
</tr>
<tr>
<td>A11</td>
<td>21</td>
<td>M</td>
<td>Yes</td>
<td>Moderate-severe</td>
<td>4.9</td>
<td>100</td>
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<tr>
<td>A12</td>
<td>55</td>
<td>M</td>
<td>Yes</td>
<td>Moderate-severe</td>
<td>2.26</td>
<td>68</td>
</tr>
<tr>
<td>A13</td>
<td>28</td>
<td>M</td>
<td>Yes</td>
<td>Moderate-severe</td>
<td>3.81</td>
<td>74.3</td>
</tr>
<tr>
<td>A14</td>
<td>39</td>
<td>M</td>
<td>Yes</td>
<td>Moderate-severe</td>
<td>2.06</td>
<td>78</td>
</tr>
<tr>
<td>A15</td>
<td>22</td>
<td>M</td>
<td>Yes</td>
<td>Moderate-severe</td>
<td>4.4</td>
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<tr>
<td>A16</td>
<td>28</td>
<td>F</td>
<td>Yes</td>
<td>Moderate-severe</td>
<td>2.3</td>
<td>73</td>
</tr>
<tr>
<td>A17</td>
<td>21</td>
<td>M</td>
<td>Yes</td>
<td>Moderate-severe</td>
<td>3.6</td>
<td>76</td>
</tr>
</tbody>
</table>

| N1      | 45  | F   | No    | None         | 4.7            | 100     | >16    |
| N2      | 20  | M   | No    | None         | 5.45           | 104     | >8     |
| N3      | 21  | M   | No    | None         | 5.05           | 100     | >8     |
| N4      | 20  | M   | No    | None         | 5.55           | 110     | >8     |
| N5      | 25  | M   | No    | None         | 3.90           | 100     | >8     |
| N6      | 33  | M   | No    | None         | 3.5            | 100     | >16    |
| N7      | 29  | F   | No    | None         | 4.64           | 100     | >8     |
| N8      | 21  | M   | No    | None         | 4.7            | 100     | >8     |
| N9      | 22  | M   | No    | None         | 5.2            | 100     | >32    |
| N10     | 19  | M   | No    | None         | 3.54           | 100     | >16    |
| N11     | 21  | F   | No    | None         | 3.0            | 78      | >16    |
| N12     | 19  | F   | No    | None         | 4.8            | 104     | >32    |
| N13     | 20  | F   | No    | None         | 5.9            | 118     | >32    |
| N14     | 21  | M   | No    | None         | 4.3            | 107     | >8     |
| N15     | 20  | M   | No    | None         | 3.6            | 105     | >8     |
| N16     | 34  | F   | No    | None         | 3.54           | 100     | >16    |
| N17     | 30  | F   | No    | None         | 4.6            | 106     | >8     |

*a Forced expiratory volume in 1 s.
Primary cultures were established by seeding freshly brushed bronchial epithelial cells into culture dishes containing 3 ml serum-free hormonally supplemented bronchial epithelium growth medium (Clontech, San Diego, CA) supplemented with 50 IU/ml penicillin and 50 μg/ml streptomycin. When confluent, the cells were passaged (p1) using trypsin and were allowed to further expand until used for experimentation at passage 2 or 3; control experiments confirmed that there was no significant difference between the responses of the cells at p2 or p3. Viability was assessed by exclusion of trypan blue dye, and the epithelial nature of cells was assessed by immunohistochemical staining of cultures grown on culture chamber slides (Labtek II eight-well chamber slides; Fisher Scientific, Loughborough, U.K.) using a pan-cytokeratin Ab as well as Abs specific for cytokeratin 13 and 18.

Cytokine release by bronchial epithelial cells

Primary bronchial epithelial cells were seeded into 24-well plates (Nunc; Life Technologies) at a density of 5 × 10^4/well and allowed to grow to 80–90% confluence. The bronchial epithelium growth medium was then replaced with basal medium (Clontech) containing insulin, transferrin, and sodium selenite supplement (Sigma-Aldrich, Poole, U.K.), and the cells were rendered quiescent for 24 h before exposure to enzymatically active extracts of house dust mite (Der p 1, 5000 U/ml) (ALK, Copenhagen, Denmark), TNF-α (Peprotech, London, U.K.) (20 ng/ml), IL-4 (20 ng/ml), or IL-13 (20 ng/ml) for 24 h, as detailed in Results. Release of IL-8, GM-CSF, or TGF-α into culture supernatants was measured by using ELISA kits according to the manufacturer’s instructions (IL-8, Pelikine Research Diagnostics, Flanders, NJ; GM-CSF, BioSource, Nivelles, Belgium; TGF-α, Oncogene Research Products, San Diego, CA). Cell number was determined by uptake of Methylene blue, and cytokine release was expressed as pg/10^6 cells.

Effect of Der p and TNF-α on IL-8 and GM-CSF release by bronchial epithelial cells

Although TNF-α and the proteolytic activity of Der p both have the potential to cause apoptosis (3), neither Der p (5000 U/ml) nor TNF-α (10 ng/ml) caused significant induction of apoptosis in the primary cultures at the concentrations used (data not shown). However, exposure of bronchial epithelial cells to Der p caused a dose-dependent increase in IL-8 and GM-CSF release by normal and asthmatic bronchial epithelial cell cultures (Fig. 2). For normal cultures, median (range) IL-8 release increased from 2.5 (0.1–8.2) to 7.7 (1.0–59.9) and 11.9 (1.7–59.7) ng/10^6 cells (n = 12), and GM-CSF increased from 101 (0–1274) to 128 (0–5358) and 173 (27–5308) pg/10^6 cells in response to 2500 and 5000 U/ml Der p, respectively; comparing the values for the asthmatic cultures were 2.9 (0.1–14.5) increasing to 11.9 (0.2–46.9) and 13.9 (0.4–37.7) ng/10^6 cells (n = 14) for IL-8, and 200 (0–798) increasing to 375 (42–1708) and 361 (29–1785) pg/10^6 cells for GM-CSF. TNF-α also significantly stimulated IL-8 and GM-CSF release from the epithelial cultures (Fig. 2). In each case, cytokine release was significantly (p < 0.05) reduced by the presence of dexamethasone, but, in the case of TNF-α, this remained significantly (p < 0.05) above basal levels. There was no significant difference in the magnitude of cytokine release by the normal and asthmatic cultures.
quiescent by growth factor removal. Under these conditions, neither IL-4 nor IL-13 had any significant effect on cell number. In the majority of subjects, exposure of bronchial epithelial cells to IL-4 caused a significant increase in IL-8 and GM-CSF release, and this was suppressible by corticosteroid treatment, irrespective of disease status (Fig. 3). However, in the IL-4-treated asthmatic epithelial cell cultures, the level of IL-8 was still significantly (p < 0.05) greater than the untreated control (Fig. 3a), even though there was significant (p < 0.01) suppression of the IL-4-induced response by dexamethasone. Although IL-13 significantly enhanced IL-8 release, the potency of IL-4 usually exceeded that of IL-13. Similarly, stimulation of GM-CSF release by IL-4 was less than that by IL-4 and failed to reach statistical significance after correcting for multiple testing. When either IL-4 or IL-13 was tested in the presence of Der p, release of GM-CSF and IL-8 was enhanced and was at least equivalent to the sum of the amounts released in the presence of either agent alone (Fig. 4).

Effect of Th2 cytokines and Der p on TGF-β release
To determine whether IL-4 and IL-13 might affect remodelling responses independently of inflammation, we tested their effects on epithelial release of the potent epithelial and fibroblast mitogen, TGF-β. Using the asthma-derived epithelial cell cultures, significant stimulation of TGF-β occurred in the presence of IL-4, IL-13, or TNF-α, and this was blocked by dexamethasone (Fig. 5a). As found for IL-8 and GM-CSF, TGF-β release was also enhanced by Der p, and this was further increased in the presence of IL-4 or IL-13 (Fig. 5b); however, in this case, the effect was always less than additive. In the 9 of 12 normal epithelial cell cultures, a small increase in TGF-β release was observed under the same treatment conditions; however, this failed to achieve statistical significance even though significant IL-8 release was observed from these cultures (Fig. 5).

Table II. Cell surface expression of IL-13Rα2 and IL-4Rα subunits on primary bronchial epithelial cells derived from nonasthmatic (n = 1–3) and asthmatic (A1–3) subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>IL-13Rα2</th>
<th>IL-4Rα</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>2.1</td>
<td>11.2</td>
</tr>
<tr>
<td>N2</td>
<td>9.1</td>
<td>24.2</td>
</tr>
<tr>
<td>N3</td>
<td>11.2</td>
<td>16.4</td>
</tr>
<tr>
<td>A1</td>
<td>3.6</td>
<td>nda</td>
</tr>
<tr>
<td>A2</td>
<td>18.0</td>
<td>33.0</td>
</tr>
<tr>
<td>A3</td>
<td>12.7</td>
<td>14.9</td>
</tr>
</tbody>
</table>

* nd, Not done.
Discussion

Bidirectional communication between the resident structural cells and the cells of the innate and adaptive immune system is essential for protection and maintenance of airway function. As the physical barrier to the external environment, the bronchial epithelium occupies a central role in this complex microenvironment by secreting a variety of proinflammatory mediators in response to environmental stimuli and by responding to mediators derived from those immune and inflammatory cells attracted into the airways (31).

Although the effects of IL-4 and IL-13 on epithelial cell lines have been reported previously (9–11), in this study, we provide the first detailed comparative analysis of the responsiveness of normal and asthmatic bronchial epithelial cells. Although experiments with cell lines tend to show reproducible changes in response to stimulation, considerable variation was observed with the primary cell cultures, both at baseline and after stimulation; this most likely reflects differences arising from the outbred human donor population. However, while individual cultures tended to vary in the amount of cytokine released, the overall responses showed consistency in that they tended to move in the same direction, and a donor who had a low response in one assay usually responded weakly in other assays.

In our study, we set out to test the hypothesis that asthmatic bronchial epithelial cells have a heightened response to an allergic environment by comparing responses to IL-4 and IL-13 that would result from the effect of allergen on immune cell function with any direct effects of allergen on epithelial responses. Significantly, even though all atopic asthmatic subjects were sensitive to house dust mite, we found no differences in cytokine release from normal or asthmatic epithelial cell cultures in response to Der p and Th2 cytokines, either alone or in combination. This suggests that for cytokine release, there is no difference in epithelial cell sensitivity to the direct effects of inhaled house dust mite allergen in asthma when compared with normal subjects and that, if present, IL-4 or IL-13 will promote IL-8 and GM-CSF release irrespective of disease status. Because all forms of asthma are associated with enhanced production of Th2 cytokines, it seems likely that provision of IL-4 and IL-13 is a key difference between asthmatic and normal subjects. This proposal is consistent with the association between atopy and asthma (32, 33) and with the occurrence of promoter and functional polymorphisms in the genes encoding IL-4 and IL-13 (13), which lead to increased cytokine production or enhanced receptor binding. However, as our experiments were performed at maximally stimulating doses of IL-4, we cannot exclude the possibility that certain single nucleotide polymorphisms in components of the IL-4R signaling cascade that are also associated
with asthma and atopy (13) may also enhance sensitivity to IL-4 when this cytokine is limiting.

The ability of IL-4 and IL-13 to augment cytokine release from bronchial epithelial cells in the presence of Der p may provide one explanation for the extent of inflammation in asthmatic Airways. Because allergen is universally inhaled, predisposition toward Th2 inflammation in asthmatic Airways will amplify epithelial release of GM-CSF and IL-8, leading to exaggerated inflammatory responses in asthma. Consistent with this role, IL-8 is a potent chemotactic agent for neutrophils and eosinophils (34, 35) and its levels are increased in asthmatic bronchial epithelium and lavage fluid (36, 37). GM-CSF promotes maturation of dendritic cells and eosinophil survival, and, in an animal model of allergic inflammation, expression of GM-CSF as a transgene in the bronchial epithelium causes persistent inflammation by preventing T cell anergy (38, 39).

In contrast with TNF-α, which gave a consistent increase in cytokine release when used as a control proinflammatory stimulus, the responses of the epithelial cell cultures to IL-4 and IL-13 were much more variable, with some cultures failing to show any response to these cytokines. Although several polymorphisms have been identified, which have positive effects on IL-4 and IL-13 signaling, this system is also tightly controlled by a variety of negative regulators. These include SHP phosphatase, silencing of cytokine signaling proteins (40–43), a dominant-negative STAT-6 splice variant whose expression we have recently described in normal and asthmatic bronchial epithelial cells (21), and IL-13Rα2 (18). The latter appears to negatively regulate IL-13 function by competing for IL-13 binding and promoting receptor internalization (17). In our study, we provide the first evidence that this receptor is expressed in human bronchial epithelium, suggesting that it may be involved in regulating epithelial responses to IL-13 and IL-4. Although we did not undertake a systematic analysis of surface IL-13Rα2 expression in each of the cultures that we studied, it is interesting that of the six individuals analyzed, the culture that expressed the highest level of IL-13Rα2 failed to respond to IL-13 and IL-4 in the face of a normal response to TNF-α (fold stimulation above baseline using IL-4, IL-13, and TNF-α as stimuli was 0.6, 0.2, and 6.0, respectively, for IL-8, and 1.1, 0.6, and 2.2 for GM-CSF). This suggests that IL-13Rα2 may be an important modifier of epithelial responses to Th2 cytokines and that further work examining the function and regulation of IL-13Rα2 expression in normal and asthmatic bronchial epithelium is warranted.

The lack of any overall significant difference between responses of normal and asthmatic bronchial epithelial cells to Der p and IL-4 or IL-13 differs from a previous study that reported that asthmatic bronchial epithelial cells are more sensitive to diesel exhaust particles when assessed by release of GM-CSF, IL-8, and RANTES (44, 45). This suggests that some epithelial responses are regulated by conditions in the airway microenvironment (e.g., the provision of Th2 cytokines), while other responses are fundamental to the nature of the asthmatic epithelium. Because we have found that asthmatic bronchial epithelial cells are more sensitive to oxidant-induced apoptosis (30), it seems likely that agents such as diesel exhaust particles, which have the capacity to generate reactive oxygen, impact on an underlying difference in the ability of asthmatic epithelial cells to deal with oxidant stress provided by environmental pollutants. However, it is also evident that Th2 inflammation has the potential to impinge on this susceptibility through stimulation of endogenous, inflammatory cell-derived oxidants.

In addition to their proinflammatory role, IL-4 and IL-13 have been implicated in goblet cell metaplasia by enhancing IL-8 release that causes oxidant-induced activation of the epidermal growth factor receptor as a consequence of attraction of neutrophils into the epithelium (46). However, as IL-4 has been shown to affect (mucin 5 subtypes A and C) expression in the absence of neutrophils in vitro (9), we explored the possibility that IL-4 and IL-13 may activate the epidermal growth factor receptor directly by increasing release of TGF-α, one of its activating ligands. Recent studies have implicated TGF-α in the process of retinoic acid-induced goblet cell metaplasia in air-liquid interface cultures grown in the presence of IL-13 (47). TGF-α release was readily detected in asthmatic cell culture supernatants, while for most of the normal cell supernatants, TGF-α levels were close to the limit of detection. Consistent with its effects in hepatocytes (48), we found that TNF-α also increased TGF-α release into asthmatic epithelial cell culture supernatants. Because neutrophils, mast cells, and macrophages are all sources of TNF-α, this finding suggests a novel mechanism whereby TNF-α can make a significant contribution to remodelling responses via TGF-α mobilization.

In contrast with IL-8 and GM-CSF, which were released with similar efficacies from normal and asthmatic epithelial cells, no
significant stimulation of TGF-α release was detected in the non-
asthmatic culture supernatants under any of the conditions tested; how-
ever, there was a trend for increased release. Failure to achieve
statistical significance probably reflects the lower levels of TGF-α
detectable in the normal epithelial cell culture supernatants and
may be due to a difference in the expression, processing, or use of
TGF-α, rather than to any selective suppression of responses to the
Th2 cytokines. Consistent with this proposal, IL-4 and IL-13 were
found to stimulate IL-8 release even though the same cultures
failed to respond with TGF-α release. TGF-α is synthesized as a
transmembrane precursor whose cleavage is catalyzed by metal-
loproteases including TNF-α-converting enzyme (49). Thus, fail-
ure to detect TGF-α in nonasthmatic culture supernatants may be
due to either low TGF-α gene expression or failure to process and
release the growth factor (or a combination of both factors).
Alternatively, as epithelial cells express epidermal growth factor re-
ceptors, TGF-α may be synthesized and cleaved equivalently by
normal and asthmatic cell cultures, but it may be used more rapidly
by the nonasthmatic cell cultures, resulting in an apparent lack of
growth factor release. Systematic analysis of each of these pro-
cesses will be required to determine the underlying cause of this
potentially important difference between the normal and asthmatic
epithelial cells. On the one hand, excessive production of TGF-α
in asthma may contribute to airway remodelling by affecting both
goblet cell metaplasia and fibroblast proliferation, whereas in con-
trast, failure of asthmatic epithelial cells to use TGF-α may un-
derlie an abnormal repair response in asthma and explain the ex-
tent of epithelial disruption that is characteristic of this disease.

Our finding that Der p enhances release of TGF-α from epithe-

cial cells has never been reported previously. Der p allergens are
known to contain cysteine and serine protease activity (50); how-
ever, it is not known whether they directly cleave the TGF-α pre-
cursor or activate cell surface metalloproteases. As many growth
factors are produced as cell surface precursors, it will be important
to determine to what extent allergen-derived proteases modify the
cell surface leading to production of biologically active molecules,
as well as causing disruption of adhesion junction proteins.

In conclusion, we have provided evidence for functional inter-
actions between allergen and Th2 cytokines in the bronchial epi-

erium in asthma linked to proinflammatory and remodelling re-


sponses. Our data identify the IL-13Rα2 as a potential regulator of
IL-13 and IL-4 signaling in these cells and suggest an important
phenotypic difference between normal and asthmatic epithelial
cells linked to TGF-α metabolism. The availability of a reliable
method for the growth of primary cultures from normal and asth-

matic bronchial epithelium should now pave the way toward dis-
section of the underlying mechanisms that control bronchial epi-

telial cell function in an allergic environment.

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