Mitotic Asynchrony Induces Transforming Growth Factor- β 1 Secretion from Airway Epithelium

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Abstract

We recently proposed that mitotic asynchrony in repairing tissue may underlie chronic inflammation and fibrosis, where immune cell infiltration is secondary to proinflammatory cross-talk among asynchronously repairing adjacent tissues. Building on our previous finding that mitotic asynchrony is associated with proinflammatory/fibrotic cytokine secretion (e.g., transforming growth factor [TGF]- β 1), here we provide evidence supporting cause-and-effect. Under normal conditions, primary airway epithelial basal cell populations undergo mitosis synchronously and do not secrete proinflammatory or profibrotic cytokines. However, when pairs of nonasthmatic cultures were mitotically synchronized at 12 hours off-set and then combined, the mixed cell populations secreted elevated levels of TGF- β 1. This shows that mitotic asynchrony is not only associated with but is also causative of TGF- β 1 secretion. The secreted cytokines and other mediators from asthmatic cells were not the cause of asynchronous regeneration; synchronously mitotic nonasthmatic epithelia exposed to conditioned media from asthmatic cells did not show changes in mitotic synchrony. We

also tested if resynchronization of regenerating asthmatic airway epithelia reduces TGF- β 1 secretion and found that pulse-dosed dexamethasone, simvastatin, and aphidicolin were all effective. We therefore propose a new model for chronic inflammatory and fibrotic conditions where an underlying factor is mitotic asynchrony.

Keywords: asthma; mitosis; transforming growth factor- β 1; fibrosis

Clinical Relevance

Current drug strategies for asthma aim for steady-state tissue exposure with long-acting glucocorticoids for convenient dosing. Our model establishes a foundation for novel treatments in asthma and other diseases targeting cellular regeneration and capture of the mitotic clock in addition to suppressing immune-mediated inflammation.

Injury to airway epithelium in humans can be caused by a variety of environmental and infectious insults and is particularly pronounced in certain disease states, such as asthma and chronic obstructive pulmonary disease (1–3). The proliferative kinetics of

airway re-epithelialization after injury are known to be important to restoration of lung homeostasis after injury (4–10). Relative to kinetics, other established mitotic behaviors (e.g., synchrony, symmetry, and ultrastructure) are

understudied, even though they are likely essential contributors to lung homeostasis and regeneration in response to injury.

Our previous work focused on mitotic synchrony in airway epithelial cell populations and showed that, although

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nonasthmatic populations progress in relative synchrony through the cell cycle (G_1 , S , G_2 , M) *in vitro*, asthmatic epithelial basal cells proliferate with a more even distribution among the cell cycle phases (i.e., mitotic asynchrony) (11). As evidence of its homeostatic importance, this asthmatic mitotic asynchrony was associated with elevated basolateral secretion of transforming growth factor (TGF)- β 1, an important mediator of lung fibroproliferation and matrix deposition (12–14), and other proinflammatory and/or profibrotic cytokines implicated in asthma pathology. These data are supported by other studies that have reported mitotic behaviors to be especially important for maintaining and restoring homeostasis in lung diseases with recurrent epithelial injury (3, 15).

The purpose of the current study was to establish the direction of the relationship between mitotic asynchrony and TGF- β 1 secretion from asthmatic airway epithelium. Our results extend and clarify our previously proposed hypothetical model that asthmatic TGF- β 1 secretion is, at least in part, downstream of intrinsic defects in epithelial mitotic behavior(s) (11, 16).

Materials and Methods

Sample Acquisition and Preparation

Nonasthmatic ($n = 10$) and asthmatic ($n = 11$) human primary bronchial airway (i.e., tracheobronchial) epithelia were obtained

commercially. Some samples ($n = 3$ nonasthmatic; $n = 3$ asthmatic) were acquired as fully differentiated epithelia on collagen IV-coated transwell membrane inserts at an air-liquid interface (#AIR-606 and #AIR-606-Asthma; MatTek Corp., Ashland, MA). The remaining samples ($n = 7$ nonasthmatic; $n = 8$ asthmatic) were obtained as epithelial basal cells (CC-2540, 1914911; Lonza Inc., Walkersville, MD). All donors underwent bronchoscopic brushings and lung biopsies to provide epithelial cells. Our cultures did not contain immune cells. The available descriptive donor information is shown in Table 1.

Cell Culture, Mechanical Injury, and Drug Exposures

For experiments at air-liquid interface, cultures were studied as we previously described (11). Briefly, they were incubated in basic medium beginning at -48 hours. Cultures were mechanically scrape-wounded in a standardized manner with a P1000 pipette tip at 0 hours and thereafter exposed continuously to bromodeoxyuridine (BrdU) (BD Bioscience, San Jose, CA). They were pulsed with mitotic synchrony-inducing compounds (i.e., 20 μ M dexamethasone (DEX) [Invitrogen, Grand Island, NY], 10 μ M simvastatin (SIM) [Sigma, Saint Louis, MO], 10 μ M aphidicolin (APH) [Sigma]), or PBS for 2 hours at -26 , -2 , $+22$, and $+46$ hours. Induced asynchrony experiments in submerged culture conditions were performed on human

bronchial epithelial cells at $< 70\%$ confluence in bronchial epithelial growth media (BEGM) with SingleQuots (Lonza, Walkersville, MD). Mitotic asynchrony was induced in parallel cultures, from the same donor, of nonasthmatic proliferating tracheobronchial epithelial cells ($n = 7$) via a 12-hour staggered exposure to basic and complete medium over 24 hours. An asynchronous population was created by combining the same number of cells from each of the parallel cultures into a mixed culture. Aliquots of each were reserved as controls before mixing the cultures. PKH67 (Sigma) was used to fluorescently label one cell population before coculture to allow tracking each population in the mixed condition. Cultures were continuously exposed to BrdU after mixing to ensure identification of the proliferating population. Cells and media were collected at 0, $+6$, $+12$, and $+24$ hours.

Flow Cytometry

Wound regeneration/mitosis was analyzed via flow cytometry for 7-AAD (eBioscience, San Diego, CA) DNA staining in BrdU $^{+}$ cells. Flow cytometry data was analyzed using Flow Jo 7.5 software (Tree Star Inc., Ashland, OR).

Cytokine Measurements

Cell culture supernatants were analyzed for cytokine secretion using the Milliplex MAP Human Cytokine/Chemokine Premixed 42 Plex (Millipore, Billerica, MA). TGF- β 1

Table 1. Donor Data

Gender	Age (yr)	Race	Medical History	Known Current Medications	Tobacco Smoke Exposed
M	2	Caucasian	Asthma	Albuterol	No
F	7	Caucasian	Asthma	Albuterol	No
F	9	African American	Asthma	Albuterol, fluticasone, salmeterol	No
F	9	African American	Asthma	Albuterol, fluticasone, salmeterol	No
F	19	Hispanic	Asthma	Albuterol, fluticasone, salmeterol	No
F	27	African American	Asthma	Unknown	No
F	43	African American	Asthma	Albuterol, fluticasone, prednisone	No
F	45	Caucasian	Asthma/COPD	Albuterol, fluticasone, salmeterol	Yes
F	46	Caucasian	Asthma	Unknown	Yes
F	59	Caucasian	Asthma/COPD	Albuterol, fluticasone, salmeterol	No
F	64	Caucasian	Asthma/COPD	Albuterol, fluticasone, salmeterol	No
M	11	Caucasian	None	None	No
M	13	Caucasian	None	None	No
M	14	African American	None	Fexofenadine	No
M	16	African American	None	None	No
F	17	African American	None	None	No
M	19	Caucasian	None	None	No
F	32	Caucasian	None	Levothyroxine, insulin, rosuvastatin	No
F	33	Caucasian	None	None	No
M	43	Caucasian	None	None	No
F	44	Caucasian	None	None	Yes

Definition of abbreviation: COPD, chronic obstructive pulmonary disease.

was measured using ELISA (Invitrogen) and/or Milliplex MAP TGF- β 1 (Millipore). Cytokines measured using the Milliplex platform were quantified and analyzed using the Luminex 200 xPONENT 3.1 system (Millipore). All samples were tested following the manufacturers' protocols.

Immunofluorescence

Immunofluorescence was performed as described in the online supplement. Fluorescence was visualized on a confocal microscope (LSM510; Zeiss, Thornwood, NY).

Statistical Analyses

Statistical comparisons were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL) and Excel 2013 (Microsoft, Redmond, WA) using *t* test functions (paired when appropriate) within time points. Cytokine data were log-transformed for inference testing. Results are reported as mean $\pm$ SEM unless otherwise noted.

Results

Mitotic Asynchrony among Neighboring Cells Is Sufficient To Induce TGF- β 1 Secretion

Our previous data on primary tracheobronchial epithelial cell cultures showed that cytokine secretion was coincident with neighboring mitotically active cells distributed broadly over the cell cycle (i.e., mitotic asynchrony) (11). We also showed that reestablishing mitotic synchrony using brief pulse glucocorticoid exposures was coincident with a reduction in secreted TGF- β 1, IL-13, and IL-1 β (11). However, we did not determine if mitotic asynchrony was necessary and sufficient to induce cytokine secretion. To test this, we used primary tracheobronchial epithelial basal cells from five unrelated nonasthmatic donors, grown submerged at a low enough density (< 70% confluence) to maintain a proliferative state throughout the experiment. Cultures from each donor were then split into two parallel cultures. One culture was grown in basic medium (BEGM with gentamycin) for 12 hours followed by 12 hours in complete medium (BEGM with SingleQuots), and the other culture was grown in 12 hours of complete medium followed by 12 hours of basic medium. The net result of this was to offset the cell cycles between the two cell culture populations (Figure 1A). The cell populations were then recultured as a mixed culture wherein neighboring cells had

Figure 1. Induced mitotic asynchrony in proliferating nonasthmatic airway epithelial basal cells stimulates transforming growth factor (TGF)- β 1 secretion. (A) Experimental design for inducing mitotic asynchrony in nonasthmatic primary airway epithelial basal cells. Mitotic asynchrony was induced in parallel submerged cultures of nonasthmatic proliferating airway epithelial basal cells via transient exposure to basic media only (for 12 h) in a staggered fashion. At 0 hours, cells from one flask were labeled with the fluorescent membrane dye PKH67. Aliquots of each were reserved as controls before mixing the cultures. Cultures were continuously exposed to bromodeoxyuridine (BrdU). Cells and media were collected at 0, +6, +12, and +24 hours. (B) Cell cycle phase was analyzed by flow cytometry for 7-AAD DNA staining in BrdU⁺ cells. At +6 hours, the mixed cultures showed asynchronous mitosis (G₁ [mean $\pm$ SEM], S, G₂/M: 47 $\pm$ 7, 22 $\pm$ 2, 31 $\pm$ 8%, respectively). Data are shown as mean $\pm$ SEM (*n* = 7 nonasthmatic donors). (C) BrdU⁺ cells as a percentage of the total number of cells in the culture at each time point according to condition. PKH67⁻ cells (i.e., basic before complete medium) proliferate more rapidly in isolation than in mixed conditions. (D) Supernatants were analyzed for TGF- β 1 at each time point and compared according to condition. The cells grown in mixed conditions show elevated TGF- β 1 secretion between +6 and +12 hours compared with either cell population in isolation. Data are shown as mean $\pm$ SEM (*n* = 5 nonasthmatic donors). **P* < 0.05.

cell cycles that were out of phase or as single populations.

The single-population cultures maintained mitotic synchrony throughout the 24-hour culture period and showed stable low-level secretion of TGF- β 1. At +6 hours, the mixed cultures showed asynchronous mitosis (G₁ [mean $\pm$ SEM], S, and G₂/M: 47 $\pm$ 7, 22 $\pm$ 2, and 31 $\pm$ 8%, respectively) (Figure 1B). This was coincident with 2-fold suppression of proliferation (i.e., [BrdU⁺] events) in cells cultured in basic medium for the first 12 hours compared with its +6-hour single-population control (Figure 1C). This was followed by up to a 4-fold increase in TGF- β 1 secretion at +12 hours that resolved by +24 hours (Figure 1D). These data suggest that offset cell cycles among neighboring cells are sufficient to induce TGF- β 1 secretion.

Mitotic Synchrony Is Not Altered by Conditioned Media from Asthmatic (Mitotically Asynchronous) Epithelial Cells

We showed previously that asthmatic airway epithelial cells are mitotically

asynchronous, and this was coincident with secretion of several cytokines (11). However, it is possible that measured or unmeasured mediators secreted into the cell culture media are capable of disrupting cell cycle synchrony. To test this, we obtained conditioned culture media from the apical (via apical rinse) and basolateral surfaces of fully differentiated primary normal and asthmatic airway epithelial cultures at air-liquid interface at several time points during active regeneration after scrape-wounding (see Figure E1 in the online supplement). Additional wounded, fully differentiated nonasthmatic cultures were exposed to these media at corresponding time points (Figure 2A) throughout regeneration. However, there was no evidence of mitotic asynchrony in cultures exposed to conditioned media from wounded asthmatic or nonasthmatic cells (Figure 2B). These data indicate that conditioned media from mitotically asynchronous airway epithelial cells is not sufficient to alter mitotic synchrony in nonasthmatic cell populations.

Figure 2. Secreted mediators do not induce mitotic asynchrony in regenerating nonasthmatic airway epithelia. (A) Experimental schema for wounded nonasthmatic (non-) epithelium exposed to conditioned media from regenerating nonasthmatic (non-) or asthmatic (asthma) airway epithelia. Nonasthmatic and asthmatic airway epithelia were cultured, and apical secretions and basolateral conditioned media were collected. New nonasthmatic epithelium was exposed apically to these secretions and basolaterally to these conditioned media as shown for the corresponding times. For example, secretions and media collected from +22 to +24 hours was used for +0 to +22 hours in the new epithelial cultures. (B) Mitotic synchrony/asynchrony according to secretion exposure. Data are shown as mean $\pm$ SEM ($n = 3$ asthmatic and 3 nonasthmatic epithelia). BrdU, bromodeoxyuridine.

Resynchronization of Asthmatic Airway Epithelial Mitosis Reduces Basolateral TGF- β 1 Secretion

Glucocorticoids are highly efficacious anti-inflammatory drugs. In addition, glucocorticoids can synchronize cell cycles across a population of cells. Two additional widely prescribed drug series, statins (HMG CoA reductase inhibitors) and tetracyclines (DNA polymerase- α inhibitors), also have cell cycle synchronizing effects. Therefore, we hypothesized that pulse-dosed glucocorticoids, statins, and tetracyclines reduce TGF- β 1 secretion via cell cycle resynchronization. To test this, fully differentiated, scrape-wounded, primary nonasthmatic and asthmatic airway epithelial cultures at air-liquid interface

were periodically exposed to the glucocorticoid DEX, the statin SIM, and the tetracycline APH. In a technique we have termed “mitotic capture,” daily 2-hour pulses of drug were given, as we previously described (11).

Without mitotic capture, asthmatic epithelial mitosis was more evenly distributed among the cell cycle phases (G_1 [mean $\pm$ SEM], S, and G_2 /M: 47 ± 4 , 24 ± 6 , and $29 \pm 6\%$, respectively) than nonasthmatic epithelial mitosis (71 ± 1 , 12 ± 2 , $17 \pm 2\%$, respectively) (Figure 3A). Mitotic analyses were limited to actively proliferating (i.e., BrdU⁺) epithelial cells. The mitotic capture of the G_1 /S checkpoint via daily 2-hour pulse drug exposure to DEX predictably increased the percentage

of asthmatic cells in G_1 (Figure 3A), thus resynchronizing their mitosis toward normal. In addition, immunofluorescence of culture cross-sections showed fewer than half of the actively mitotic cells (Ki67⁺) adjacent to the wound in asthmatic compared with nonasthmatic epithelia. Mitotic capture with DEX increased the abundance of Ki67⁺ cells 4-fold adjacent to the wound and 10-fold distant from the wound (Figure E2). DEX-induced resynchronization of mitosis in the proliferating asthmatic epithelia resulted in a concurrent reduction in basolateral TGF- β 1 secretion, in particular at the 24-hour peak from 285 ± 74 μ g/ml to 45 ± 17 μ g/ml (Figure 3B). The DEX-induced improvement in mitotic synchrony (increased % of mitotically active cells in G_1) correlated with a reduction in basolateral TGF- β 1 secretion at 24 hours (Figure 3C).

The mitotic capture of the G_1 /S checkpoint via daily 2-hour pulse drug exposure to SIM or APH, using the same donors, similarly increased the percentage of asthmatic cells in G_1 (Figure 4A). SIM- or APH-induced resynchronization of mitosis in the proliferating asthmatic epithelia resulted in a concurrent reduction in basolateral TGF- β 1 secretion, in particular at the 24-hour peak. Mitotic capture decreased TGF- β 1 at 24 hours after wounding from 285 ± 74 μ g/ml to 81 ± 26 μ g/ml with SIM and to 77 ± 50 μ g/ml with APH ($P < 0.01$ for both) (Figure 4B). DEX, SIM, and APH did not have a detectable impact on any of these parameters in nonasthmatic tracheobronchial epithelium.

Discussion

Our prior work established that proliferating airway epithelial cells are a source of TGF- β 1 and other inflammatory and/or fibrotic mediators when their mitosis is poorly synchronized (e.g., in asthma) (11). In the current study, we extended and clarified this association using a series of mitotic behavior experiments wherein airway epithelial mitosis is desynchronized by disease state or experimental condition and then resynchronized via manipulation of the G_1 /S checkpoint. These experiments resulted in three core findings. (1) Inducing mitotic asynchrony in proliferating nonasthmatic airway epithelial basal cells is sufficient to stimulate TGF- β 1 secretion; (2) asthmatic

Figure 3. Asthmatic epithelial mitotic asynchrony and transforming growth factor (TGF)- β 1 secretion are improved by dexamethasone (DEX). (A) Mean mitotic synchrony/asynchrony in *in vitro* airway epithelia at air-liquid interface after exposure to PBS or DEX. (B) Basolateral TGF- β 1 secretion according to compound and time after wounding. (C) Correlation between DEX-induced improvement in mitotic synchrony (% of mitotically active [BrdU]⁺ cells in G₁) and reduction (%) in basolateral TGF- β 1 secretion at 24 hours. Data are shown as boxplots with median, interquartile range, and 95% confidence intervals. (n = 8 asthma and 3 nonasthmatic donors). $\bullet$ represents ≥ 1 standard deviation from the median; * represents ≥ 2 standard deviations from the median. $^{\#}P < 0.01$.

epithelial secreted TGF- β 1 and other mediators do not alter mitotic synchrony in regenerating injured nonasthmatic airway epithelia; and (3) resynchronization of asthmatic airway epithelial mitosis via mitotic capture reduces basolateral TGF- β 1 secretion. Cumulatively, these analyses show that mitotic synchrony is the homeostatic state in airway epithelial basal cell populations and that poorly -synchronized mitosis (as in asthma) induces TGF- β 1 secretion.

The proliferative characteristics of airway epithelial basal cells, as the cells responsible for airway re-epithelization, are likely important for restoring homeostasis in diseases of chronic or recurrent epithelial injury, such as asthma. However, we have shown previously (11) and have validated herein that the basal cells in asthma undergo mitosis in a poorly synchronized

manner. This runs counter to the normal homeostatic state where peaks and troughs in central circadian cortisol rhythms synchronize peripheral (organ/tissue-level) oscillators, which in turn synchronize mitosis within those organ systems (17). Airway epithelial basal cells are relatively quiescent under normal conditions, with infrequent mitosis (18). Quantified in mice, airway epithelial cells turnover slowly ($< 1\%$ per 24 h) (19) until stimulated to proliferate by injury ($> 10\%$ per 24 h) (20). Our prior work and that of others has shown this to be similarly true in human *in vitro* airway epithelium (11, 21).

Perturbation of central and peripheral circadian oscillators has been shown to lead to profibrotic wound healing phenotypes and altered cell proliferation characteristics (11, 22). BMAL1 and CLOCK are the major peripheral oscillators that act via

dimerization and nuclear translocation. There they increase transcription of the cryptochrome and period genes by binding to their E box promoter regions (23). In the absence of BMAL1 (i.e., BMAL1 knockout mice), experimentally reduced arterial blood flow simulating atherosclerotic disease resulted in vascular remodeling with a thicker vessel wall and increased collagen deposition compared with wild type (24). In addition, femoral artery injury in mice deficient in CLOCK or BMAL1 led to fibrotic wound healing and vascular wall thickening compared with wild type (24). Furthermore, CLOCK mutant and BMAL1 knockout mice are characterized by smaller pancreatic islets due to decreased cell proliferation (25).

In this context and based on our findings, it is reasonable to hypothesize that restoration of central and/or peripheral oscillator functions would synchronize asynchronous basal cell mitosis. In fact, there have been recent promising developments of so-called “chronotherapy” agents for several diseases, including rheumatoid arthritis (26, 27). The chronotherapeutic concept used in our experiments, which we termed “mitotic capture,” is to temporarily pause the cell cycle at a specific point every 24 hours, allowing lagging mitotic cells to catch up before releasing them all to progress synchronously through mitosis. Specifically, we resynchronized asthmatic airway epithelial mitosis via mitotic capture using the following reversible cell cycle inhibitors: (1) DEX, a synthetic glucocorticoid derivative of cortisol, activates the G₁/S checkpoint of the cell cycle via p53-dependent upregulation of p21 (28). p21

Figure 4. Asthmatic epithelial mitotic asynchrony and transforming growth factor (TGF)- β 1 secretion are improved by simvastatin (SIM) and aphidicolin (APH). (A) Mitotic synchrony/asynchrony in *in vitro* airway epithelia at air-liquid interface after exposure to PBS, SIM, or APH. (B) Basolateral TGF- β 1 secretion according to compound and time after wounding. Data are shown as boxplots with median, interquartile range, and 95% confidence intervals (n = 8 asthma and 3 nonasthmatic donors). $\bullet$ represents ≥ 1 standard deviation from the median; * represents ≥ 2 standard deviations from the median. $^{\#}P < 0.05$ versus PBS.

then binds the cyclin-cyclin-dependent kinase (CDK) 2 complex, preventing it from completing RB protein phosphorylation and progression into S phase (29). (2) SIM is a HMG-CoA reductase inhibitor that similarly activates the G₁/S checkpoint via inhibition of p21 and p27 degradation, in turn preventing the cyclin E/CDK2 activity (30). (3) APH is an antimicrobial drug that reversibly inhibits DNA polymerase- α preventing DNA synthesis, thereby preventing progression into the synthesis (S) phase of mitosis (31). APH has been shown to induce the release of pre-formed cytokines from cell granules (31, 32), potentially accounting for the induction of TGF- β 1 seen at 0 hours in our experiments.

Reduced basolateral TGF- β 1 secretion as a result of mitotic capture with these agents is suggestive of a cause-and-effect relationship between mitotic asynchrony and the secretion of TGF- β 1. However, DEX, SIM, and APH are all multipotent and our observations could merely be the result of independent drug effects. One might question the choice of multipotent drugs, in addition to DEX, for our mitotic capture experiments because there are many compounds that act more specifically to arrest cell cycling. However, SIM and APH were selected for three characteristics important to successful mitotic capture: (1) the mitotic capture agent must be rapidly reversible to allow for a temporary pause in cell cycling; (2) because we previously published mitotic capture efficacy for DEX, agents that similarly act at the G₁/S checkpoint but by different mechanisms of action are informative; and (3) drug classes that are already in use in humans are most rapidly translatable for treatment. DEX, SIM, and APH meet all of these criteria. In addition, cells were exposed to the drugs in 2-hour daily pulses, so their off target effects would be limited to some degree.

To deal with the issue of drug multipotency, we incorporated two additional sets of experiments to support the argument of cause-and-effect. In the first of these, we used a novel method that we developed to show that inducing mitotic

asynchrony in normal proliferating airway epithelial basal cells is sufficient to stimulate TGF- β 1 secretion. We also determined whether mitotic asynchrony is intrinsic to the basal cell population or the result of secreted factors. We show that asthmatic epithelial secretions do not alter mitotic synchrony in regenerating injured nonasthmatic airway epithelia. There is contextual evidence for this in the literature wherein some cell types (e.g., fibroblasts, heart tissue explants and vascular epithelium) have cell-autonomous mitotic clocks that are passed to daughter cells (24, 33, 34).

Taken together, our findings establish that the lack of normal mitotic synchrony in regenerating asthmatic airway epithelium drives secretion of TGF- β 1 and not vice versa. This might be explained by differential expression of ligands and receptors in each cell cycle phase. As a hypothetical example, TGF- β 1 receptors may be expressed only during M-phase, whereas TGF- β 1 may only be expressed during S-phase. Therefore, in a synchronous population of cells, the receptor would never see the ligand. However, in an asynchronous population, some cells would be expressing the receptor coincidentally with other cells expressing the ligand.

Our use of the term “mitotic asynchrony” was originally derived from the fungal field (35). Although they are very different biological systems, the similarities are striking (e.g., synchronous regulation of multiple adjacent nuclei). In multinucleated fungi, cells maintain synchronous mitosis via passage of mitotic factors from nucleus to nucleus through the cytoplasm (35). Although our system is not technically multinucleated, the cells (and their nuclei) are connected via epithelial junctions that are known to be disrupted in asthma (36). We suspect that the inability to pass factors from cell to cell could also be a contributing factor to the lack of synchrony in asthmatic epithelium.

This is an important new concept in asthma because basolateral secretion of TGF- β 1 is an important contributor to

lung fibrosis and airway remodeling (12, 37, 38), which is characterized by recurrent epithelial damage, fibroblast proliferation and subepithelial fibrosis, and goblet cell and airway smooth muscle hyperplasia, all contributing to a continuous decline in lung function (39, 40). Furthermore, the number of epithelial or submucosal cells expressing TGF- β 1 in asthma correlates with basement membrane thickness and fibroblast number (41).

This study advances our understanding of the relationship between mitotic behaviors and airway repair; however, we recognize that there are some limitations. The use of commercial sources of cells limits the amount of clinical information that is available for each donor. We were also limited by donor availability, which makes age, gender, and race matching of nonasthmatic and asthma donors difficult. Although some of these experiments were conducted in fully differentiated cells at air-liquid interface, they do not incorporate the effects underlying airway tissues may have on epithelial mitotic behaviors.

The clinical ramifications of TGF- β 1 production as a result of asynchronous mitosis are substantial. Current drug strategies for asthma aim for steady-state tissue exposure with long-acting glucocorticoids for convenient dosing. Although steady-state exposure is effective for immune cell suppression, this strategy does not capture the tissue-level peripheral oscillator and thereby mitosis. Instead, driving mitotic synchrony requires circadian-like fluctuations in tissue steroid levels (i.e., tall peak followed by deep trough to pause and release mitosis). Chronotherapy akin to our *in vitro* experiments could be beneficial in asthma to simulate the pause in cell cycle and then synchronous release. Our model establishes a foundation for novel treatments in asthma and other diseases, targeting cellular regeneration and capture of the mitotic clock in addition to suppressing immune-mediated inflammation. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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