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(71) **Applicant** *(for all designated States except US):* **THE CHILDREN'S RESEARCH INSTITUTE** [US/US]; 111 Michigan Ave., NW, Washington, DC 20010 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only):* **FREISHTAT, Robert, J.** [US/US]; 8408 Pittsfield Court, Potomac, MD 20854 (US). **HOFFMAN, Eric, P.** [US/US]; 3910 Warner Street, Kensington, MD 20895 (US).

(74) **Agent: GREENBAUM, Michael, C ;** BLANK ROME LLP, 600 New Hampshire Ave., NW, Washington, DC 20037 (US).

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(54) Title: METHODS FOR DIAGNOSING AND TREATING ASTHMA

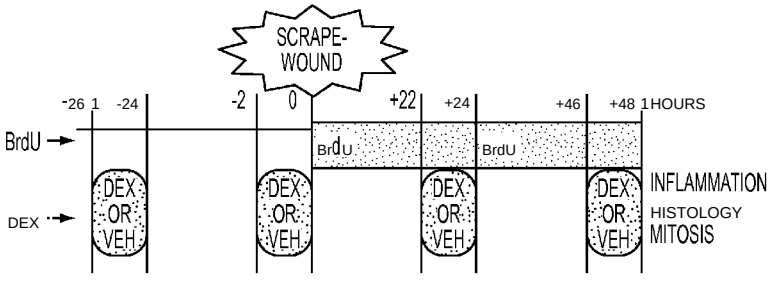

FIG. 1

(57) Abstract: The present invention relates to asthma. Particularly, the present invention relates to clinical screening, diagnosis, prognosis, therapy and prophylaxis, as well as for drug screening and drug development for the treatment of asthma. The present invention relates to a new paradigm in diagnosing, screening, and treating asthma by affecting airway epithelial synchronization.

METHODS FOR DIAGNOSING AND TREATING ASTHMA

[001] This application claims the priority of U.S. Provisional Patent Application Serial No. 61/321,411, filed April 6, 2011, which is incorporated herein by reference.

FIELD OF THE INVENTION

[002] The present invention relates to asthma. More particularly, the present invention relates to clinical screening, diagnosis, prognosis, therapy and prophylaxis, as well as for drug screening and drug development for the treatment of asthma.

BACKGROUND OF THE INVENTION

[003] Asthma is a chronic inflammatory disease of the lower respiratory tract characterized by airway hyperresponsiveness and mucus obstruction (Busse et al., Am J Respir Crit Care Med 2004, 170:683-690). Bronchial asthma is the most common chronic disease affecting children and young adults and is a complex genetic disorder with several overlapping phenotypes (Cookson and Moffatt, Hum. Mol. Genet. 9: 2359-64 (2000); Weiss, Ann. Allergy Asthma Immunol., 87 (Suppl 1): 5-8 (2001)). There is strong evidence for a genetic component in asthma (Bleecker et al., Am J Respir. Crit. Care. Med., 156: SI 13-6 (1997); Kauffmann et al., Chest, 121(3 Suppl): 27S (2002)). Multiple environmental factors are also known to modulate the clinical expression of asthma as well as the asthma-associated phenotypes: bronchial hyperresponsiveness, atropy and elevated IgE (Koppelman et al., Eur. Resp. J, 13: 2-4 (1999); Cookson, Nature, 25: B5-11 (1999); HoUoway, Clin. Exp. Allergy, 29: 1023-1032 (1999)). It is a commonly held view that asthma is caused by multiple interacting genes, some having a

protective effect and others contributing to the disease pathogenesis, with each gene having its own tendency to be influenced by the environment (Koppelman et al., 1999; Cookson, 1999; Holloway, et al., 1999). Thus, the complex nature of the asthma phenotype, together with substantial locus heterogeneity and environmental influence, has made it difficult to uncover factors that underlie asthma.

[004] Pharmacologic analogues of Cortisol (e.g. prednisone) have been used clinically since 1948 and remain the standard of care for the treatment of a variety of inflammatory diseases including asthma (Larj et al., Chest 2004; 126: 138S-149S). These glucocorticoids (GC) reduce pathological inflammation that is central to asthma, and they are thought to control clinical asthma symptoms through their anti-inflammatory effects (Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allerg y Clin Immunol 2007, 120:S94-138.). For example, Martinez and co-workers report that inhaled fluticasone shows sustained (albeit reversible) improvement in the proportion of asthma episode-free days, a reflection of reduced inflammation, compared to placebo over a two-year study period (Guilbert et al., N Engl J Med 2006, 354:1985-1997). Curiously, anti-inflammatory agents that specifically target inflammatory cells (e.g. eosinophils, T and B cells) and their intercellular signaling pathways have not shown similar efficacy to GCs in human trials (Lemanske, Proc Am Thorac Soc 2009, 6:312-315). That argues against the idea that asthmatic inflammation is merely the result of interactions between external stimuli and classic inflammatory cells like eosinophils and T cells. Rather, it is likely to involve complex interactions among multiple cell types including non-inflammatory resident cells of the lung (i.e. airway epithelium, fibroblasts, and smooth muscle).

[005] Therefore, there remains a need for a new model of asthma and the use of that model for diagnosis, drug screening, and treating asthma.

SUMMARY OF THE INVENTION

[006] To that end, the present inventors proposed a model for asthma placing airway epithelium at the center of a network of interacting inflammatory mediators. Due to its ability to simultaneously respond to airborne pathogens and environmental challenges and interact with its tissue environments, airway epithelium is regarded as a key lung tissue in asthma. In addition, airway epithelium communication with lamina propria fibroblasts (Davies et al., J Allergy Clin Immunol 2003; 111:215-225; quiz 226) and smooth muscle has been described (Malavia et al., Am J Respir Cell Mol Biol 2009;41:297-304). Our model predicts that asthmatic inflammation is driven by intrinsic inflammatory, fibrogenic, and regenerative characteristics of epithelium that are rescued by GCs.

[007] The present inventors have discovered that mitotically active asthmatic airway epithelium is asynchronous when compared to normal cells (non-asthmatic). Additionally, when those asthmatic cells are treated with a composition capable of pausing cell cycle, such as dexamethasone, the asthmatic cells become more synchronous.

[008] Accordingly, the present invention relates to methods for diagnosing asthma. The methods comprise obtaining a cell sample of an individual's airway epithelium, inducing the cells to undergo mitosis, and determining the synchrony of the mitotic cells. Asynchronous mitosis indicates the increased likelihood of asthma. Cell synchrony of the sample is compared to that of normal cells. Generally, less than about 70 percent, preferably 65 percent, more preferably 60 percent of cells in the same phase of the cell cycle indicates asynchrony and indicates an increased susceptibility to asthma.

[009] The present invention further provides methods for monitoring the treatment efficacy of an individual with asthma. The methods comprise administering a pharmaceutical

composition to an individual, obtaining a cell sample of an individual's airway epithelium, inducing the cells to undergo mitosis, and determining the synchrony of the mitotic cells. If the cells become more synchronous upon the administration of the pharmaceutical composition, the treatment is likely effective.

[0010] The present invention further provides methods for screening for an agent capable of alleviating asthma. This method involves inducing an airway epithelium cell sample to undergo mitosis, exposing the cell sample to an agent, and determining the synchrony of the mitotic cells. If the cells become increasingly synchronous upon exposure to the agent (when compared to cells not exposed to the agent), the agent is a good candidate for further study in treating asthma.

[001 1] The present invention also relates to methods for synchronizing airway epithelia by administering to the epithelia a composition capable of pausing mitosis in a particular phase of mitosis so that the cells can be synchronized. The composition preferably stops mitosis only briefly to let the cells catch up to the particular phase of mitosis. Once the cells are in phase, they can proceed through the cell cycle synchronously.

[0012] The present invention also relates to methods for treating or alleviating the symptoms of asthma in an individual by administering to the individual a composition capable of pausing mitosis in a particular phase of mitosis so that the cells can be synchronized. The composition needs to stop mitosis only briefly to let the cells catch up to the particular phase of mitosis. Once the cells are in phase, they are then allowed to proceed through the cell cycle synchronously. Thus, the composition is administered for only a short period of time (about 2 hours or less, preferably about 1 hour or less), rather than around the clock as in the current treatments of asthma which treat inflammation rather than cell synchronization.

[0013] The compositions appropriate to synchronize airway epithelia and to treat asthma include glucocorticoids, statins, azoles, and antineoplastic agents. Examples of glucocorticoids include hydrocortisone, cortisone acetate, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, beclometasone, fludrocortisone acetate, deoxycorticosterone acetate, and aldosterone. Examples of statins include atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin. Examples of azoles include clotrimazole, posaconazole, ravuconazole, econazole, ketoconazole, voriconazole, fluconazole, itraconazole, and carbimazole. Examples of antineoplastics include actinomycins such as dactinomycin; anthracyclines such as, doxorubicin, daunorubicin, valrubicin, idarubicin, and epirubicin; and certain antibiotics such as bleomycin, plicamycin, and mitomycin.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 is a chart that shows the experimental design for in vitro wounding of respiratory epithelia. Epithelia were pulsed for 2h every 24h with 20nM dexamethasone (DEX) or vehicle (VEH) at the times shown. Mechanical scrape-wounding occurred at 0h with continuous apical and basolateral BrdU exposure until cell harvest at +48h for histological and mitotic analyses. Media samples were frozen before measurement of inflammatory cytokines.

[0015] Figure 2 is a chart that shows that asthmatic epithelial basolateral secretions are relatively inflammatory after wounding. Levels of specific cytokines (TGF-βI, IL-10, IL-6, IL-13, and IL-Iβ) measured by cytometric bead assay are shown for basolateral epithelial secretions from asthmatic and normal epithelia at 0, +24, and +48h. Asthmatic epithelia had significantly higher basolateral secretion of TGF-βI, IL-10, IL-13, and IL-Iβ at one or more time points. DEX pulses decreased secretion of TGF-βI and IL-13 in asthmatic epithelia. Data are shown as mean+SEM in pg/mL.

[0016] Figure 3 is a series of charts that show that mitosis is diminished in asthmatic epithelium and increased by pulse DEX. Flow cytometry was used to measure the presence of BrdU in single cell suspensions of normal and asthmatic human airway epithelia at +48h. A) The quantity of BrdU positivity is shown as the percent of total counted cells in non-wounded, wounded, and wounded DEX-pulsed epithelial cultures. Asthmatic epithelial wounds (n=6) showed approximately 40% fewer mitotically active cells than normal epithelial wounds (n=3) but intermittent DEX exposures abrogated this difference. B) Shown are gating and cell cycle analyses for one representative wounded normal and one representative wounded asthmatic epithelial culture. All events measured by the cytometer were gated on the BrdU+FLICA- population (left panels). The selected cells were further gated by 7-AAD height and area to

remove cell doublets (middle panels). Finally, 7-AAD height was used to identify the cell cycle distribution of the gated cell population (right panel). C) Compared to wounded normal epithelia, wounded asthmatic epithelia at +48h showed a more even distribution of BrdU+ cells among the cell cycle phases (i.e. G1/G0, S, and G2/M) consistent with mitotic dyssynchrony. Intermittent exposures of the asthmatic epithelia to DEX improved cell cycle synchrony as shown by normalization of the percentage of mitotic cells in each cell cycle phase.

[0017] Figure 4 are images and a chart that show that regeneration of asthmatic human airway epithelia is impaired. A) Shown are contrast-enhanced bright-field microscopy (16X) images of scrape-wounded primary differentiated human airway epithelia at wounding (i.e. Oh) and +48h from representative normal and asthmatic donors. Normal epithelial wounds showed complete healing in all conditions (i.e. 20nM DEX or VEH) by +48h. Similarly cultured asthmatic epithelia showed thinly repaired wounds at +48h regardless of DEX exposure. [N.B. The wounds are pale appearing X-shaped regions. Thick appearing dark areas (arrows) are heaped up areas of epithelium resulting from the scraping process. B) Percent wound area reduction over 48h according to culture condition and DEX exposure. Wound area was measured in triplicate by a single operator blinded to the culture conditions using ImageJ Software.

[0018] Figure 5 are charts that show the proposed model for glucocorticoid efficacy in asthma. Tobacco smoke and viruses are among many agents known to induce apoptosis in airway epithelium, prompting regenerative processes. A) Untreated asthmatic airway epithelium is characterized by dyssynchronous regeneration that ineffectively repairs apoptotic regions of epithelium. The concomitant basolateral inflammatory cytokine secretion (e.g. increased IL-Iβ and TGF-βI, variable IL-10) would lead to pathological immune cell recruitment/activation as well as fibroblast and smooth muscle cell proliferation. B) In our proposed model for

glucocorticoid efficacy in asthma, intermittent glucocorticoid dosing simultaneously mediates anti-inflammation in injured asthmatic epithelium and increases the ability of asthmatic epithelium to synchronize its mitosis. This leads to more effective regeneration of injured regions.

[0019] Figure 6 is a chart that shows that asthmatic epithelial mitosis is dyssynchronous and is improved by both dexamethasone (DEX) and simvastatin (SIM). Cell cycle analysis was performed by flow cytometry of regenerating (i.e. BrdU+) epithelial cells from wounded cultures. Compared to wounded normal epithelia, wounded asthmatic epithelia showed a more even distribution of BrdU+ cells among the cell cycle phases (i.e. G1/G0, S, and G2/M) consistent with mitotic dyssynchrony. Exposure of the asthmatic epithelia to DEX and SIM improved cell cycle synchrony as shown by normalization of the percentage of mitotic cells in each cell cycle phase.

[0020] Figure 7 is a chart that shows that SIM and DEX effectively reduce postwounding asthmatic epithelial basolateral inflammatory secretions. Levels of cytokines measured by cytometric bead assay are shown for basolateral secretions from asthmatic and normal in vitro airway epithelia at 0, +24, and +48 hours. Data are shown as mean ± SEM in pg/mL. *P<0.05 vs. VEH

[0021] Figure 8 is a chart that shows that asthmatic epithelial apical secretions are no more inflammatory than normals after wounding. Levels of specific cytokines (IL-Iβ, IL-6, IL-10, IL-13, and TGF-βI) measured by cytometric bead assay are shown for apical epithelial secretions from asthmatic and normal epithelia at 0, +24, and +48h. Apical asthmatic epithelial cytokine secretion was not statistically significantly different from normal epithelial apical secretion. DEX-exposure decreased secretion of IL-13 in both normal and asthmatic epithelia.

Levels of other measured cytokines were unchanged on DEX-exposure, except IL-1β which differed between DEX-exposed asthmatic and normal epithelia. This difference appeared only at +24h. Data are shown as mean+SEM. Only significant (<0.05) P-values are shown for between-group comparisons (i.e. asthma versus normal) by repeated measures general linear.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0022] The present inventors have discovered that mitotically active asthmatic airway epithelium is asynchronous when compared to normal cells (non-asthmatic). Additionally, when those asthmatic cells are treated with a composition capable of pausing cell cycle, such as dexamethasone, the asthmatic cells become more synchronous.

[0023] Methods of the present invention depend on the measure of cellular synchrony. "Synchronous," or variations thereof, as used herein, refers to a population of cells where the cells are in the same phase of the mitotic cell cycle. Conversely, "asynchronous" or "dyssynchonous" or variations thereof, as used herein refers to a population of cells where the cells are in different phases of the mitotic cell cycle. Thus, a population of cells is 60 percent synchronous when 60 percent of the cells are in the same phase of the cell cycle. That same population can also be described as 40 percent asynchronous. Cellular synchrony can be determined using methods well-known in the art as cell cycle analysis. Flow cytometers are often used for cell cycle analysis. In this measurement, the relative fraction of sample cells in the G1/G0, S, G2, or M phase of the cell cycle can be determined by staining them with a DNA-specific dye and passing them through the excitation volume of a flow cytometer. The size and amount of DNA in the nucleus of a given particle is dependent on its cell cycle stage and, hence, the pulses produced by particles in different stages have different shapes. Pulses may be analyzed according to their amplitude, area, and width using well-known techniques as described in Wersto et al. (Cytometry 46:296-306 (2001)), which is incorporated herein by reference. Alternative techniques for pulse shape analysis are described in U.S. Patent Nos. 4,021,1 17 and 3,938,038, and U.S. Patent Application Publication No. 2011/0063602, which are incorporated herein by reference.

[0024] Alternatively, cell cycle analysis can be carried out using a BrdU label. That method includes: causing a BrdU to be taken into a cell for a given period; and subsequently, carrying out immunohistochemistry by using an anti-BrdU antibody. Another method for cell cycle analysis is disclosed in U.S. Patent Application Publication No. 2010/0100977, which is incorporated herein by reference.

Use of Airway Epithelium Synchrony as Diagnostics

[0025] As described herein, cellular synchrony of airway epithelia may be used as diagnostic markers for the detection, diagnosis, or prognosis of asthma. For instance, an airway epithelium sample from a patient may be assayed by any of the methods described herein, or by any other method known to those skilled in the art, for cell cycle synchrony. Asynchronous airway epithelium indicates asthmatic conditions or increased likelihood of asthma in the patient. Generally, less than about 70 percent, preferably 65 percent, more preferably 60 percent of cells in the same phase of the cell cycle indicates asynchrony and indicates an increase susceptibility to asthma.

[0026] Alternatively, the synchrony of the sample can be compared to that of a control (non-asthmatic cells). If the synchrony of the sample is less than that of the control, then asthma can be diagnosed. The diagnosis can be made by looking at airway epithelium synchrony alone or in conjunction with the other diagnostic methods known in the art, such as medical history, family history, symptoms, spirometry, methacoline challenge test, exhaled nitric oxide test, etc.

Use of Airway Epithelium Synchrony for Drug Screening

[0027] According to the present invention, airway epithelium synchrony may be used as markers to evaluate the effects of a candidate drug or agent on treating asthmatic patients.

[0028] A patient suffering from asthma is treated with a drug candidate and the progression of the disease is monitored over time by looking at his/her airway epithelium synchrony. This method comprises treating the patient with a drug candidate, periodically obtaining airway epithelium samples from the patient, determining the cellular synchrony of the samples, and comparing the synchrony over time to determine the effect of the agent on the progression of asthma. The drug candidate can be considered effective in treating asthma, if it improves cell synchrony in the patient.

[0029] Alternatively, the screening of the drug candidate can be accomplished ex vivo by using an asthmatic airway epithelia cell suspension or cell culture. Here, the cells are induced to undergo mitosis. The drug candidate is then brought into contact with the cells for a predetermined time period, preferably for less than about 4 hours, more preferably less than about 2 hours, and most preferably less than about 1 hour. More preferably, the drug candidate is administered to the cells for two periods, once within a 24 hour cycle. For example, the cells can be brought in contact with the drug candidate for 4 hours our of each 24 hour period for two periods. After that contact time, the synchrony of the cells is determined. This is then compared with a control cell population that has not been in contact with the drug candidate. If the drug candidate is able to synchronize the cells, when compared to the control, then it is a viable candidate for further testing as a drug to treat asthma.

[0030] The candidate drugs or agents of the present invention can be, but are not limited to, proteins, peptides, small molecules, vitamin derivatives, as well as carbohydrates. In addition to the proteins, DNA encoding these proteins, antibodies to these proteins, peptide fragments of these proteins or mimics of these proteins may be introduced into the patient as candidate agents. "Mimic" as used herein refers to the modification of a region or several regions of a peptide molecule to provide a structure chemically different from the parent peptide but topographically and functionally similar to the parent peptide. A skilled artisan can readily recognize that there is no limit as to the structural nature of the candidate drugs or agents of the present invention.

Use of Airway Epithelium Synchrony for Monitoring Disease Progression

[0031] Airway epithelium synchrony can also be used to monitor progression of asthma in a patient, for instance, the development of asthma. For instance, a sample from a patient may be assayed by any of the methods described herein, and the cell synchrony may be compared to the levels found in non-astmatic individuals. The airway epithelium synchrony can be monitored over time to track progression of asthma in the patient. The present methods are especially useful in monitoring disease progression because the degree of asynchonicity is proportional to the severity of asthma. Comparison of the cell synchrony may be done by researcher or diagnostician or may be done with the aid of a computer and databases.

Treatment of Asthma by Affecting Cell Synchrony

[0032] In an embodiment, the present invention provides methods for synchronizing airway epithelia by contacting the airway epithelia with a compound or drug capable of pausing mitosis (and thereby synchronizing the epithelia). The contact of the compound or drug with the

epithelia takes place over a relatively short period of time, preferably about 4 hours, more preferably about 2 hours or less, most preferably about 1 hour or less. That short contact period is sufficient to synchronize the cells. As a consequence of cellular synchronization, the airway epithelia reduces inflammatory cytokine secretion.

[0033] Cell synchrony can be used as a target for asthma treatment. Compounds or drugs that are capable of synchronizing airway epithelium can be administered to an asthmatic patient to treat, alleviate, or ameliorate symptoms of asthma. Preferably, the drug pauses mitosis in a particular phase so that the cells can be synchronized. The composition needs to stop mitosis only briefly to let the cells catch up to the particular phase of mitosis. Once the cells are in phase, the effect of the drug is no longer needed. Thus, the advantage of targeting cell synchrony, rather than inflammation, is that the drug can be used at a much lower dose because of the short time frame required to synchronize the cells. Accordingly, the dosage used is half, preferably 1/3, more preferably 1/4 of the normal recommended dosage of that particular drug, which results in lower possible side and adverse effects associated with the particular drug. Alternatively, the compound or drug can be administered or compounded so that the area under the blood concentration vs. time curve (AUC) is lower than for the recommended dosage for the particular drug. Either way, the shorter exposure to the drug results in lower side effects while maintaining effectiveness.

[0034] The compounds or drugs appropriate to synchronize airway epithelia and to treat asthma include glucocorticoids, statins, azoles, and antineoplastic agents. Examples of glucocorticoids include hydrocortisone, cortisone acetate, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, beclometasone, fludrocortisone acetate, deoxycorticosterone acetate, and aldosterone. Examples of statins

include atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin. Examples of azoles include clotrimazole, posaconazole, ravuconazole, econazole, ketoconazole, voriconazole, fluconazole, itraconazole, and carbimazole. Examples of antineoplastics include actinomycins such as dactinomycin; anthracyclines such as, doxorubicin, daunorubicin, valrubicin, idarubicin, and epirubicin; and certain antibiotics such as bleomycin, plicamycin, and mitomycin. One particular class of molecules is the compound disclosed in U.S. Patent Application Publication No. 2010/0087408, which is incorporated herein by reference.

[0035] The terms "preventing" or "treating" or "ameliorating" and similar terms used herein, include prophylaxis and full or partial treatment. The terms may also include reducing symptoms, ameliorating symptoms, reducing the severity of symptoms, reducing the incidence of the disease, or any other change in the condition of the patient, which improves the therapeutic outcome.

[0036] The administration of the drug can be through any known and acceptable route. Such routes include, but are not necessarily limited to, oral, via a mucosal membrane (e.g., nasally, via inhalation, rectally, intrauterally or intravaginally, sublingually), intravenously (e.g., intravenous bolus injection, intravenous infusion), intraperitoneally, and subcutaneously. Administering can likewise be by direct injection to a site (e.g., organ, tissue) containing a target cell (i.e., a cell to be treated). Furthermore, administering can follow any number of regimens. It thus can comprise a single dose or dosing of the drug, or multiple doses or dosings over a period of time. Accordingly, treatment can comprise repeating the administering step one or more times until a desired result is achieved. In embodiments, treating can continue for extended periods of time, such as weeks, months, or years. Those of skill in the art are fully capable of

easily developing suitable dosing regimens for individuals based on known parameters in the art. The methods thus also contemplate controlling, but not necessarily eliminating, asthma. The preferred route of administration in accordance with the present invention is via inhalation.

[0037] The amount to be administered varies depending on the subject, stage of the disease, age of the subject, general health of the subject, and various other parameters known and routinely taken into consideration by those of skill in the medical arts. As a general matter, a sufficient amount of the drug will be administered in order to make a detectable change in the symptom of asthma. Suitable amounts are disclosed herein, and additional suitable amounts can be identified by those of skill in the art without undue or excessive experimentation. The dosage used in accordance with the present invention is lower than the usual recommended dosage for the particular drug as noted in the package insert, prescribing information, or the Physician's Handbook. For the present invention, the dosage used is half, preferably 1/3, more preferably 1/4 of the normal recommended dosage of that particular drug. Alternatively, the drug can be administered so that the AUC is lower than for the recommended dosage.

[0038] The drug is administered in a form that is acceptable, tolerable, and effective for the subject. Numerous pharmaceutical forms and formulations for biologically active agents are known in the art, and any and all of these are contemplated by the present invention. Thus, for example, the drug can be formulated in oral solution, a caplet, a capsule, an injectable, an infusible, a suppository, a lozenge, a tablet, a cream or salve, an inhalant, and the like. It should be evident that the preferred dosage form provides for efficient contact of the drug with the airway epithelia to effect synchronization of those cells.

[0039] Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and use the present

invention and practice the claimed methods. The following examples are given to illustrate the present invention. It should be understood that the invention is not to be limited to the specific conditions or details described in the examples.

EXAMPLE 1

[0040] We proposed a model placing airway epithelium at the center of a network of interacting inflammatory mediators Our model predicts that asthmatic inflammation is driven by intrinsic inflammatory, fibrogenic, and regenerative characteristics of epithelium that are rescued by glucocorticoids.

[0041] We present herewith data that support this proposed model. We utilized a well-established *in vitro* system wherein human primary airway epithelial cells, lacking inflammatory cells, from normal and asthmatic individuals are differentiated at an air-liquid interface to morphologically mimic conducting airway epithelium. Our experiments showed that when induced to regenerate, asthmatic epithelium is intrinsically inflammatory, fibrogenic, and mitotically dyssynchronous. Furthermore, intermittent glucocorticoid exposures simultaneously reduced asthmatic inflammation and resynchronized epithelial mitotic regeneration.

Materials and Methods

Cell Culture and Intermittent glucocorticoid Exposures

[0042] Normal (n=3) and asthmatic (n=6) primary differentiated human airway (i.e. bronchial) epithelia grown in 12-well plates on collagen-coated Transwell membrane inserts at an air-liquid interface were obtained commercially (#AIR-606 and #AIR-606-Asthma; MatTek Corporation, Ashland, MA). Donors underwent bronchoscopic brushing to acquire epithelial

cells. Descriptive donor information provided by MatTek Corporation for the individuals from whom cells were obtained is shown in Table 1.

Table 1. Description of human bronchial epithelial cell donors[4]

	Donor Age (Years)	Gender	Race	Smoking	Medications
Asthmatic	7	Female	Caucasian	No	Albuterol
	9	Female	African American	No	Albuterol, Fluticasone, Salmeterol
	27	Female	African American	No	Unknown
	43	Female	African American	No	Oral and inhaled steroids
	45	Female	Caucasian	Yes	Albuterol, Fluticasone, Salmeterol
	46	Female	Caucasian	Yes	None
Normal	5	Female	Caucasian	No	None
	13	Male	Caucasian	No	None
	33	Female	Caucasian	No	None

[4]Provided by MatTek, Inc.

[0043] On arrival, the *in vitro* epithelia were washed with phosphate buffered saline (PBS) and the basal medium was replaced with proprietary defined medium supplied by the manufacturer. Cells were equilibrated at 37°C and 5% $C0_2$ for 16h followed by medium replacement with identical proprietary medium lacking glucocorticoids and epidermal growth factor (EGF). This condition was maintained for an additional 22h. Upon completion (i.e. -26h on the experimental timeline shown in Figure 1, intermittent glucocorticoid exposures began: Dexamethasone (DEX) (20nM) or PBS vehicle (VEH) was added to the apical and basolateral epithelial surfaces for 2h. At -24h, the medium was replaced with glucocorticoid- and EGF-free medium. This two-hour DEX/VEH pulse was repeated every 24h (i.e. at -2, +22, and +46h) thereafter until cell harvest at +48h.

Mechanical injury model

[0044] An *in vitro* epithelial injury model that allows for the study of epithelial repair processes in the lung was adapted for use in this study. Briefly, at Oh on the timeline in Figure 1, epithelia were scraped in two perpendicular lines with a p i 000 pipette tip and placed in bromodeoxyuridine (BrdU)-containing (10μM) medium. BrdU-containing medium was changed at +24h following the DEX/VEH pulse mentioned previously. Epithelia were incubated at 37°C and 5% $C0_2$ until +48h. In some experiments, wounds were imaged daily (i.e. 0, +24, +48h) using a 16X phase contrast objective lens and wound area was measured in triplicate by a single operator blinded to the culture conditions using ImageJ Software (Rasband. ImageJ. Bethesda, Maryland, USA: U. S. National Institutes of Health; 1997-2009). Contrast was enhanced equally in all images to improve wound visualization.

[0045] Although the cultures were kept at an air-liquid interface, depending on interval culture time they generated up to 0.5 mL of apical secretions (including fluid used to wash the

apical surface). These apical secretions and all basolateral media (~lmL) were collected at all time points and frozen prior to analysis. No medium was added to the apical surface of the cultures to maintain them at an air-liquid interface.

Analysis of Inflammatory Mediators

[0046] Inflammatory (i.e. interleukin [ILJ-Iβ, IL-6, IL-10, and IL-13) and fibrogenic (i.e. transforming growth factor [TGFJ-βI) cytokines were measured in apical and basolateral secretions at 0, +24, and +48h by flow cytometry on a FACSCalibur™ System (BD Biosciences, San Jose, CA) using a FlowCytomix Multiplex Kit with FlowCytomix Pro 2.3 software (Bender MedSystems, Burlingame, CA). These cytokines were selected as an initial screening set for these experiments because of their prominent role in asthmatic inflammation and/or remodeling

Cell Cycle Analysis

[0047] Epithelia were washed once with PBS and harvested at +48h (Figure 1) for analysis by flow cytometry. A single cell suspension was achieved by exposure for 5 minutes with trypsin - ethylenediaminetetraacetic acid (EDTA) solution (#T3924; Sigma-Aldrich, St. Louis, MO) followed by filtration through a 40 μιη strainer. Cells were simultaneously labeled with the following according to the manufacturers' protocols: 1) Carboxyfluorescein FLICA Apoptosis Poly-Caspase Detection Kit (Immunochemistry Technologies, LLC, Bloomington, MN) and 2) APC BrdU Flow Kit containing 7-AAD (amino-actinomycin-D) (BD Biosciences, San Jose, CA). Flow cytometry data were generated on a FACSCalibur™ System (BD Biosciences). Samples were gated to study 7-AAD content in $BrdU^{+}FLICA^{-}$ cells. Data were analyzed by means of the cell cycle analysis feature of FlowJo 7.6 (Tree Star, Inc., Ashland, OR) using a Watson (Pragmatic) model with equal coefficients of variation for the G1/G0 and G2/M peaks

Statistical Analysis

[0048] Statistical comparisons were performed in SPSS 17.0 software (SPSS Inc., Chicago, IL) using T-test functions within time points. Results are reported as mean+SEM unless otherwise noted.

Results

Injured Asthmatic Epithelium is Inflammatory and Fibrogenic

[0049] Flow cytometric bead assays were used to quantify a select screening group of inflammatory (i.e. IL-1β, IL-6, IL-10, and IL-13) and fibrogenic (i.e. TGF-β1) cytokines in apical and basolateral secretions from asthmatic and normal epithelia at 0, +24, and +48h post-wounding. Normal and asthmatic epithelia at time 0h (i.e. before wounding) exhibited statistically similar levels of the cytokines investigated except for higher basolateral secretion of IL-10. However, asthmatic epithelia basolaterally secreted significantly higher levels of four of the five cytokines during wound healing (Figure 2). In particular, there was a significant between-group (i.e. asthma > normal) difference for secretion of TGF-β1, IL-10, IL-13, and IL-1β (all $P<0.05$) for at least one time point during wound healing (Figure 2). IL-6 was the only cytokine for which basolateral secretion was not significantly different between asthmatic and normal epithelia. With DEX pulses, asthmatic TGF-β1 and IL-13 basolateral secretion were significantly reduced. Although generally higher than in basolateral secretions, cytokine levels in apical secretions were not different between wounded untreated asthmatic and normal epithelia, except that IL-1β levels were significantly increased at 24h in asthmatic secretions (Figure 8).

Asthmatic Epithelial Cell Mitosis is Slow and Dyssynchronous

[0050] Epithelia were harvested into single cell suspensions at +48h for flow cytometry. Non-wounded asthmatic and normal epithelia showed similar minimal background levels of $BrdU^+$ cells, an indicator of mitosis (Figure 3A). Due to the lack of mitotic cells, no cell cycle analysis was performed on samples from this condition.

[0051] Wounded asthmatic epithelia showed 40% fewer $BrdU^+$ cells than wounded normal epithelia (mean+SEM: 0.32+0.05% vs. 0.56+0.07% of total cells; P=0.03). Exposure of normal cells to pulses of DEX did not significantly alter the quantity of $BrdU^+$ cells in normal epithelia, whereas the quantity of asthmatic epithelial mitosis approximated normal levels with DEX pulses (0.55+0.13%; P=0.19 vs. wounded untreated asthmatic cells) (Figure 3A).

In order to evaluate normal and asthmatic epithelial mitosis during wound repair, flow cytometric cell cycle analysis for DNA content (i.e. 7-AAD) was performed by gating on $BrdU^+$ cells with no detectable caspase activation (i.e. apoptosis) (Figure 3B). Notably, $caspase^+$ (i.e. apoptotic) cells were rare in all conditions. Cells in active mitosis were presumed to be regenerating the scrape wound because of the extremely low rate of background mitosis in non-wounded cultures. As shown in Figure 3C, normal epithelial mitosis was fairly synchronous (e.g. >70% of cells in G1/G0) in the absence and presence of pulse DEX. Conversely, mitotically-active asthmatic epithelial cells exhibited a dyssynchronous distribution among the cell cycle phases (i.e. G1/G0, S, G2/M) (53+5, 21+3, 26+4%) compared to normal epithelia (71+1, 12+2, 17+2%). DEX-pulsed asthmatic cells showed similarly synchronous mitotic activity to normal cells.

Normal and Asthmatic Epithelia Exhibit Differential Wound Healing

[0052] Wounded normal and asthmatic epithelia were imaged by bright field microscopy daily at 0, +24, and +48h using a 16X phase contrast objective lens. As illustrated in Figure 4A,

normal epithelial wounds showed visible healing regardless of DEX pulse. Alternatively, the asthmatic scars appeared relatively thin and were still visible at +48h regardless of DEX pulses. This was evaluated quantitatively by measurement of the wound area. Normal wound area decreased from 0 to +48h by 78.6+7.7% with vehicle alone and 86.8+5.4% with DEX pulses. However, in the absence of DEX, asthmatic epithelial wound area decreased by significantly less (50.2+7.5%; P=0.02) than normals. With DEX pulses, asthmatic wound narrowing improved significantly (75.7+9%; P=0.04) (Figure 4B).

Discussion

[0053] We studied cultures of human primary differentiated asthmatic and normal airway epithelia cultured at an air-liquid interface. As in a recent study (Parker et al., *Pediatr Res* 67:17-22), we found that confluent, quiescent normal and asthmatic epithelial cultures were similar with minimal secretion of cytokines and mitotic activity as evidenced by BrdU labeling. However, upon mechanical wounding, asthmatic and normal epithelia exhibited different responses. The asthmatic epithelial cultures showed increased basolateral secretion of inflammatory/fibrogenic cytokines (as exemplified by TGF-βI, IL-10, IL-13, and IL-Iβ) and showed slow, poorly synchronized mitosis relative to normal controls. This predictably was associated with the poor wound repair observed for asthmatic epithelia. Those markers of inflammation and dyssynchronous regeneration were attenuated by intermittent glucocorticoid-pulses. These results support our proposed model that predicts asthmatic inflammation is driven by intrinsic inflammatory, fibrogenic, and regenerative characteristics of airway epithelium that are rescued by glucocorticoids.

[0054] Cytokine (i.e. TGF-βI, IL-10, IL-13, and IL-Iβ) secretion in our experiments in response to epithelial injury is important given accumulating evidence for airway epithelium-induced inflammatory cell recruitment (Cheng et al., / Immunol 2007, 178:6504-6513; Hammad et al., Nat Rev Immunol 2008, 8:193-204), and proliferation of fibroblasts (Perng et al., Am J Respir Cell Mol Biol 2006, 34:101-107; Hostettler et al., Clinical & Experimental Allergy 2008, 38:1309-1317; Royce et al., Annals of Allergy, Asthma and Immunology 2009, 102:238-246) and smooth muscle (Malavia et al., Am J Respir Cell Mol Biol 2009, 41:297-304). In particular, basolateral secretion of TGF-βI, which was increased in asthmatic epithelia in our experiments, is one of the key mediators of fibroblast and smooth muscle proliferation (Makinde et al., Immunol Cell Biol 2007, 85:348-356) and is a central component of our previously published airway epithelial stress response gene/protein network (Freishtat et al., J Investig Med 2009). Further, IL-Iβ activates many inflammatory genes in asthma (Rosenwasser, J Allergy Clin Immunol 1998, 102:344-350) and IL-13 is a critical mediator of the classical Th2 asthmatic inflammation (Walter et al., J Immunol 2001, 167:4668-4675; Wills-Karp, Respiratory Research 2000, 1:19-23). Conversely, IL-10 is a potent immunoregulatory and anti-inflammatory cytokine that suppresses eosinophils (Takanaski et al., J Exp Med 1994;180:711-715), decreases airway hyperresponsiveness (Makela et al., PNAS 2000, 97:6007-6012; Justice et al., Am J Physiol Lung Cell Mol Physiol 2001, 280:L363-368), and is increased during acute viral exacerbations of asthma (Grissell et al., Am J Respir Crit Care Med 2005;172:433-439). The elevated basolateral secretion of IL-10 from asthmatic epithelium at all time points suggests a constitutive epithelial counter-regulation of inflammation *in vitro.* This runs counter to reports of decreased IL-10 in BAL fluid from individuals with asthma (Borish et al., The Journal of allergy and clinical immunology 1996, 97:1288-1296; Message et al., PNAS 2008, 105:13562-13567).

However, this difference may be accounted for by BAL fluid cytokines reflecting both apical epithelial and inflammatory cell secretions.

[0055] In addition to inflammation, epithelial regeneration is of particular importance in asthma due to the fact that many typical asthma triggers, including tobacco smoke and viruses, are known to induce apoptotic injury in airway epithelium (Tesfaigzi et al., Am J Respir Cell Mol Biol 2006, 34:537-547). Epithelial stress/injury, independent of inflammation, has been observed in moderate and severe childhood asthma (Fedorov et al., Thorax 2005, 60:389-394). In fact, airway epithelial injury, in the forms of physical damage to the columnar cell layer and apoptosis (Cohen et al., Am J Respir Crit Care Med 2007, 176:138-145; Bucchieri et al., Am J Respir Cell Mol Biol 2002, 27:179-185), is a hallmark of asthma (Holgate, Allergol Int 2008, 57:1-10). Puchelle and colleagues have shown that regeneration of normal human airway epithelium in response to injury includes three stages: cell spreading/migration, proliferation, and differentiation (Zahm et al., Cell Motil Cytoskeleton 1997, 37:33-43; Puchelle et al., Proc Am Thorac Soc 2006, 3:726-733). Using a similar in vitro mechanical injury model to the one used in our study, Wadsworth et al showed that normal human differentiated airway epithelial wounds closed over the initial 16 to 24h. This primarily reflected cell migration mediated by autocrine EGF secretion that subsequently led to mitosis of epithelial cells within the wound (Wadsworth et al., J Clin Immunol 2006;26:376-387). Our results for normal epithelium wound repair were temporally similar to those described by Wadsworth et al. However, the thinly repaired wounds in asthmatic cultures observed in our study are consistent with effective migration without effective regeneration.

[0056] The simultaneous resolution of inflammation and resynchronization of epithelial mitotic regeneration on exposure to intermittent glucocorticoids following in vitro injury

addresses an important inconsistency in asthma. That is, despite well-demonstrated anti-inflammatory efficacy, inhaled glucocorticoids have not been shown to improve long-term pathologic airway remodeling. The classic model is that asthmatic inflammation leads to long-term pathological lung function decline (i.e. remodeling). However, this is not supported by several trials that have shown inhaled glucocorticoids improve lung function in the short-term but regresses toward the placebo group over the course of several years (Guilbert et al., N Engl J Med 2006, 354:1985-1997; The Childhood Asthma Management Program Research Group, N Engl J Med 2000, 343:1054-1063; Murray et al., Lancet 2006;368:754-762). Recently, this was confirmed by a large trial comparing inhaled budesonide to placebo in children and adults with recent-onset mild persistent asthma. In this study, the initial pre- and post-bronchodilator differences in fraction of expired volume in 1 second (FEV_1) between the treatment and placebo groups disappeared by the fourth year of the study (Busse et al., J Allergy Clin Immunol 2008, 121:1167-1174). Further, asthmatic bronchial biopsy reticular layer thickness does not decrease with inhaled glucocorticoid treatment unless given at relatively high doses (Sont et al., Am J Respir Crit Care Med 1999, 159:1043-1051). These patients were only studied for 2 years so any sustained long-term effect of relatively high dose glucocorticoids remains unclear. Therefore, our data address this inconsistency in asthma by supporting our proposed alternative model of glucocorticoid efficacy in asthma shown in Figure 5. Therein, direct anti-inflammatory effects of intermittent glucocorticoid dosing are accompanied by simultaneous resynchronization of epithelial mitosis thereby reducing pathological lung remodeling in asthma.

[0057] Pulsatile secretion of endogenous adrenal glucocorticoids (i.e. Cortisol in humans) can reset an organism's internal and peripheral circadian clocks (Knutsson et al., J Clin Endocrinol Metab 1997, 82:536-540), and this has been shown to occur in the bronchiolar

epithelium where it is mediated by Clara cells (Gibbs et al., Endocrinology 2009, 150:268-276). The result of this is synchronous progression of a tissue's cells through normal regeneration/mitosis. The intermittent glucocorticoid exposure scheme used in our experiments was a gross reflection of a circadian peak in circulating endogenous glucocorticoid levels. Although crude by comparison to in vivo glucocorticoid circadian fluctuations, a 2 hour pulse glucocorticoid exposure is sufficient to induce precursors to the inhibition of mitosis, including cyclin-dependent kinase inhibitor $p57^{kip2}$ (Puddicombe et al., Am J Respir Cell Mol Biol 2003, 28:61-68) and clock gene Perl (Balsalobre et al., Science 2000, 289:2344-2347).

[0058] In summary, these data, generated in an airway model lacking inflammatory cells, support the concept that asthmatic epithelium is intrinsically inflammatory, fibrogenic and mitotically dyssynchronous. These results support our previously proposed model predicting asthmatic inflammation is driven by intrinsic inflammatory, fibrogenic, and regenerative characteristics of epithelium that are rescued by glucocorticoids (Freishtat et al., *Journal of Investigative Medicine* 2010, 58:19-22). If extended by further studies, anti-inflammatory treatment of asthma with glucocorticoids may best be redirected to target pathological lung remodeling directly.

EXAMPLE 2

[0059] Fundamental questions persist about the pathobiology underlying asthma. A prime example of this is the paradigm on which the standard of care for asthma, anti-inflammationwith glucocorticoids, is based: chronic asthmatic inflammation is the upstream impetus for long-term airway remodeling (e.g. goblet cell hyperplasia, lung function decline, basement membrane thickening). However, this model is called into question by the persistence of airway remodeling despite effective anti-inflammation with glucocorticoids. To address this inconsistency, we proposed that the principal target of current asthma treatment regimens, inflammation, is actually downstream of the causal biological defect, remodeling (Figure 5). We showed in Example 1 above that human primary differentiated asthmatic airway epithelial inflammatory cytokine secretions correlate with dyssynchronous mitosis upon in vitro mechanical injury. We show in this example that improving asthmatic airway epithelial cell mitotic cell cycle synchrony reduces inflammatory cytokine secretion.

Materials and Methods

[0060] Human fully-differentiated (air-liquid interface) normal (n=3) and asthmatic (n=3) primary airway epithelia, lacking inflammatory cells, were cultured in glucocorticoid-free medium beginning at -48h. The cells were pulsed with mitotic cell cycle synchrony-inducing (i.e. dexamethasone, simvastatin) compounds or vehicle for 2h at -26, -2, +22, and +46h. Cultures were mechanically scrape-wounded at Oh and thereafter exposed continuously to bromodeoxyuridine (BrdU) to identify mitotically active cells. The time line for the experiment is shown in Figure 1.

Results

[0061] The results confirmed our previous findings, discussed in Example 1, that asthmatic epithelia secreted more basolateral cytokines and regenerated less efficiently than normals following wounding (Figure 6). Asthmatic epithelia were dyssynchronously distributed along the cell cycle (G1/G0, S, G2/M: 52+10, 25+4, 23+7%) compared to normal epithelia (71+1, 12+2, 17+2%) (Figure 6). Dexamethasone pulses improved mitotic cell cycle synchrony (72+5, 8+2, 20+4%) (Figure 6) while reducing asthmatic epithelial inflammatory cytokine secretion (Figure 7). Similarly, simvastatin improved asthmatic epithelial mitotic cell cycle synchrony (75+6, 11+4, 14+3%) (Figure 6) and reduced basolateral TGFB1, IL-6, and IL-13 secretion ($0.01<P<0.04$) (Figure 7).

[0062] Although certain presently preferred embodiments of the invention have been specifically described herein, it will be apparent to those skilled in the art to which the invention pertains that variations and modifications of the various embodiments shown and described herein may be made without departing from the spirit and scope of the invention. Accordingly, it is intended that the invention be limited only to the extent required by the appended claims and the applicable rules of law.

What is claimed is

1. A method for diagnosing asthma in an individual comprising the steps of

a. obtaining a cell sample of an individual's airway epithelium;

b. inducing the cells to undergo mitosis; and

c. determining the synchrony of the mitotic cells, wherein asynchronous mitosis indicates the increased likelihood of asthma.

2. The method of claim 1, wherein less than about 70 percent of cells in the same phase of the cell cycle indicates asynchrony.

3. A method for monitoring a treatment efficacy of an individual with asthma comprising the steps of

a. administering a candidate compound to an individual;

b. obtaining a cell sample of an individual's airway epithelium;

c. inducing the cells to undergo mitosis; and

d. determining the synchrony of the mitotic cells, wherein the candidate compound is effective in the treatment of asthma if the cells become more synchronous after the administration of the pharmaceutical composition.

4. The method of claim 3, further comprising the step of determining the release of inflammatory cytokines by the cells.

5. The method of claim 3, wherein the candidate compound is selected from the group consisting of proteins, peptides, small molecules, vitamin derivatives, and carbohydrates.

6. The method of claim 3, wherein the dosage of the candidate compound used in step a is lower than the recommended dosage for the candidate compound.

7. The method of claim 3, wherein the dosage of the candidate compound used in step a is about 0.5 times the recommended dosage for the candidate compound.

8. A method for screening for an agent capable of alleviating asthma comprising the steps of

a. inducing an airway epithelium cell sample to undergo mitosis;

b. contacting the cell sample with an agent; and

c. determining the synchrony of the mitotic cells, wherein the agent is a candidate for treating asthma when the cells are increasingly synchronous after exposure to the agent.

7. The method of claim 6, further comprising the step of determining the release of inflammatory cytokines by the mitotic cells.

8. The method of claim 6, wherein the agent is selected from the group consisting of proteins, peptides, small molecules, vitamin derivatives, and carbohydrates.

9. The method of claim 6, wherein step b lasts two hours or less.

10. A method for treating asthma in an individual comprising the step of administering to the individual a composition capable of pausing mitosis in a particular phase so that mitotic airway epithelial cells can be synchronized.

11. The method of claim 10, wherein the composition is a glucocorticoid, a statin, an azole, or an antineoplastic agent.

12. The method of claim 11, wherein the glucocoricoid is hydrocortisone, cortisone acetate, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, beclometasone, fludrocortisone acetate, deoxycorticosterone acetate, or aldosterone.

13. The method of claim 11, wherein the statin is atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, or simvastatin.

14. The method of claim 11, wherein the azole is clotrimazole, posaconazole, ravuconazole, econazole, ketoconazole, voriconazole, fluconazole, itraconazole, or carbimazole.

15. The method of claim 11, wherein the antineoplastic agent is actinomycins such as dactinomycin; anthracyclines such as, doxorubicin, daunorubicin, valrubicin, idarubicin, and epirubicin; and certain antibiotics such as bleomycin, plicamycin, or mitomycin.

16. The method of claim 10, wherein the dosage of the composition is lower than the recommended dosage for the composition.

17. The method of claim 10, wherein the dosage of the composition is about 0.5 times the recommended dosage for the candidate compound.

18. A method for synchronizing airway epithelia comprising the step of contacting the airway epithelia with a compound capable of pausing mitosis in a particular phase.

19. The method of claim 18, wherein the compound is a glucocorticoid, a statin, an azole, or an antineoplastic agent.

20. The method of claim 18, wherein the contacting step lasts two hours or less.

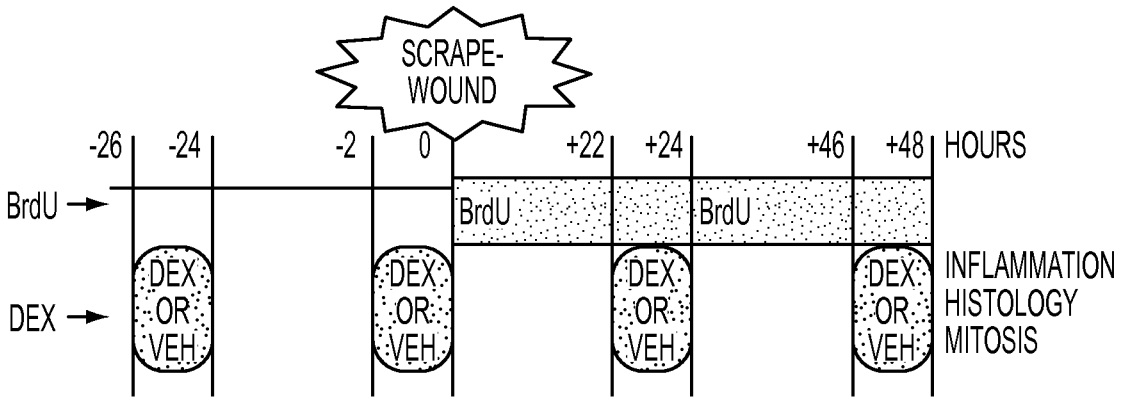
SCRAPE-
WOUND
-26
-24
-2
0
+22
+24
+46
+48
HOURS
BrdU
DEX
BrdU
BrdU
DEX OR VEH
DEX OR VEH
DEX OR VEH
DEX OR VEH
INFLAMMATION
HISTOLOGY
MITOSIS

FIG. 1

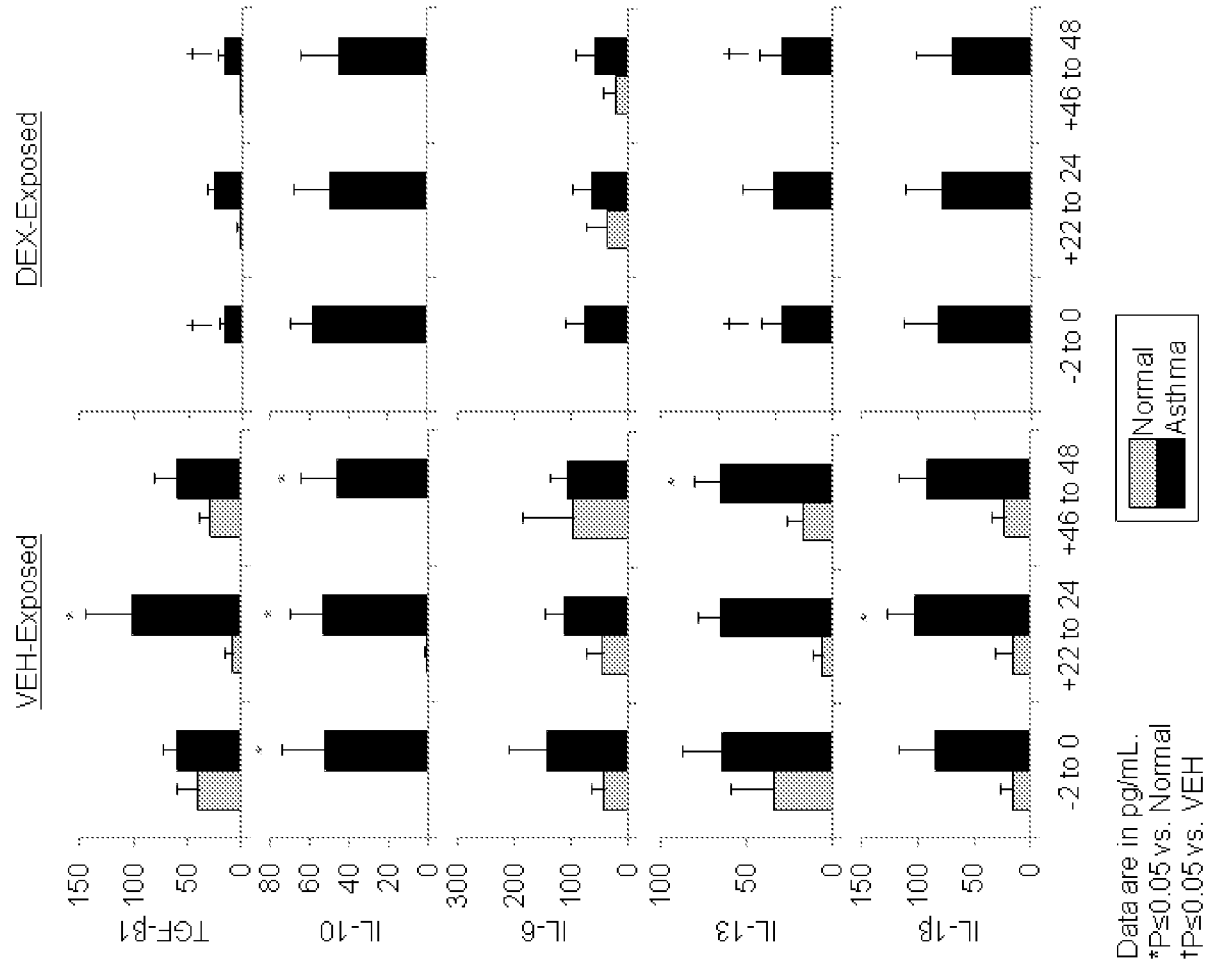
VEH-Exposed
DEX-Exposed
TGF-β1
IL-10
IL-6
IL-13
IL-1R
-2 to 0
+22 to 24
+46 to 48
-2 to 0
+22 to 24
+46 to 48
Normal
Asthma
Data are in pg/mL.
*P≤0.05 vs. Normal
†P≤0.05 vs. VEH

FIGURE 2

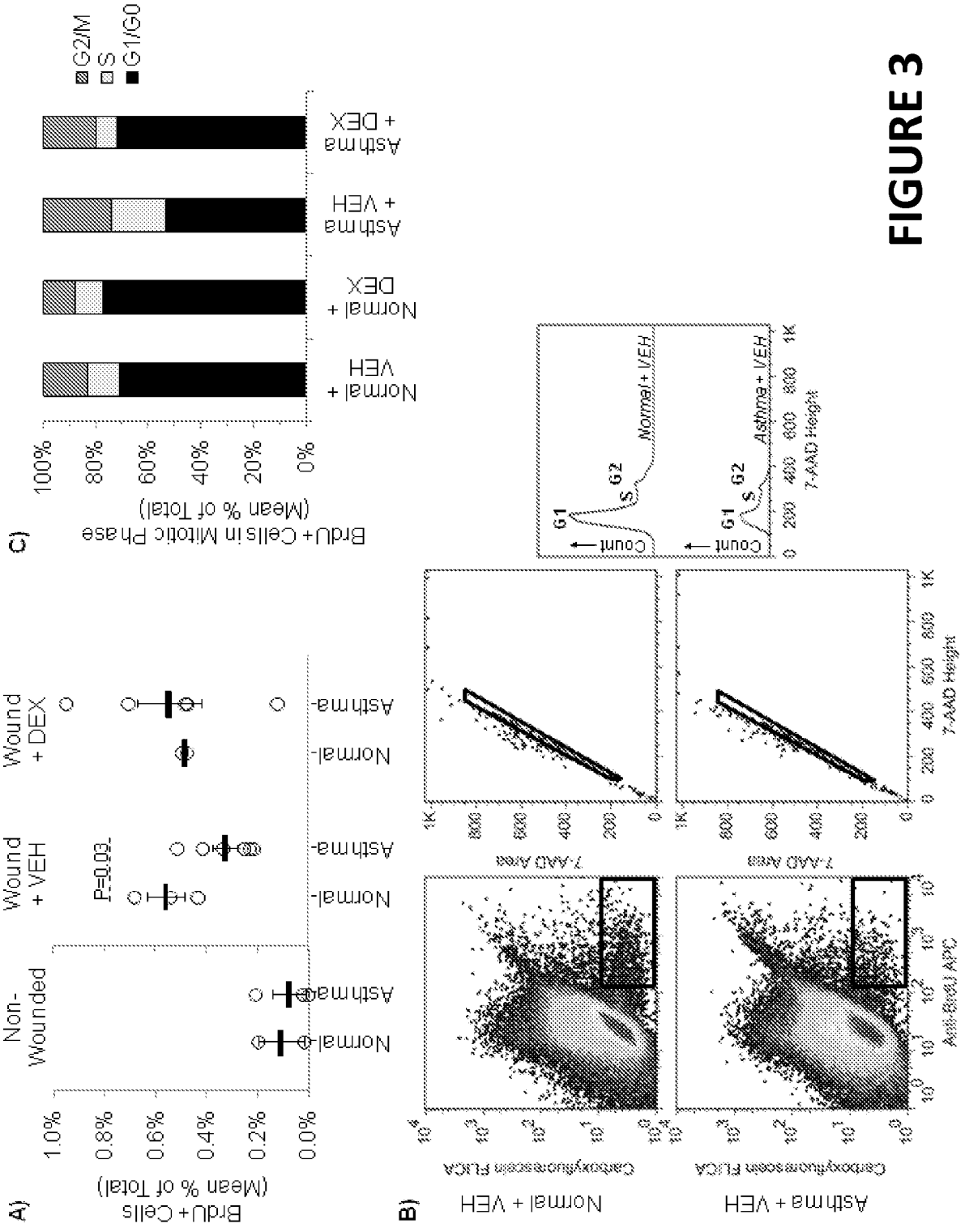
A)
BrdU+ Cells (Mean % of Total)
1.0%
0.8%
0.6%
0.4%
0.2%
0.0%
Non-Wounded
Wound + VEH
Wound + DEX
P=0.03
Normal-
Asthma-
B)
Normal + VEH
Asthma + VEH
Carboxyfluorescein FLICA
Anti-BrdU APC
7-AAD Area
7-AAD Height
G1
S
G2
Count
Normal+ VEH
Asthma+ VEH
C)
BrdU+ Cells in Mitotic Phase (Mean % of Total)
100%
80%
60%
40%
20%
0%
G2/M
S
G1/G0
Normal + VEH
Normal + DEX
Asthma + VEH
Asthma + DEX

FIGURE 3

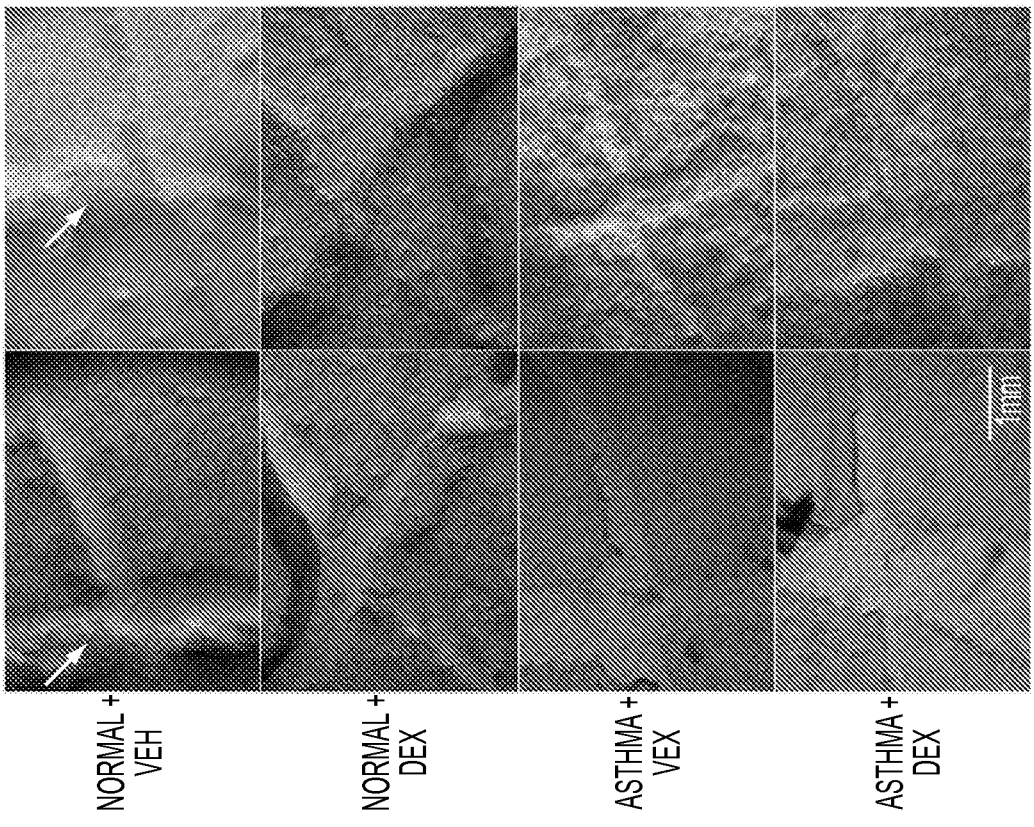
0
+48 HOURS
NORMAL + VEH
NORMAL + DEX
ASTHMA + VEX
ASTHMA + DEX
1mm

FIG. 4A

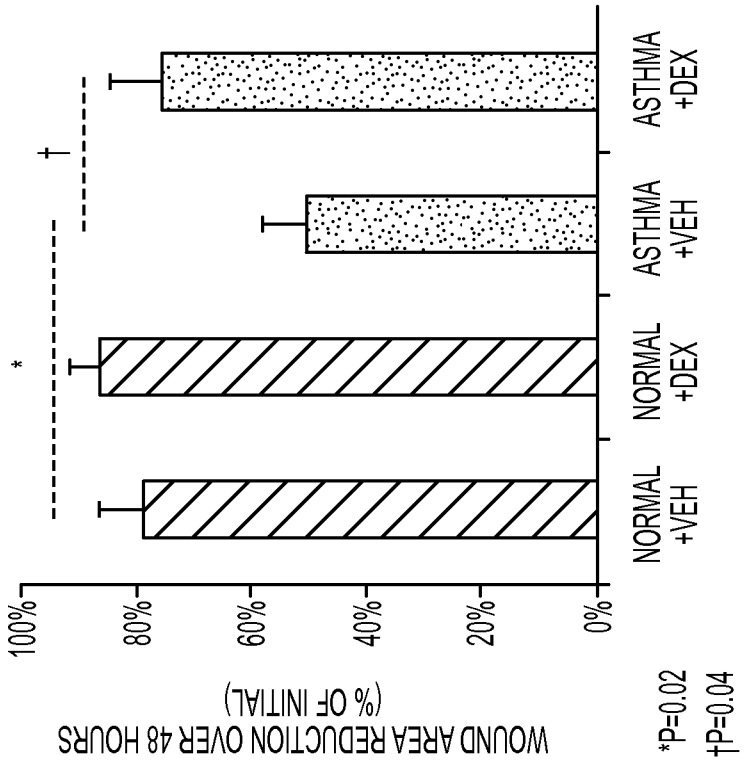
WOUND AREA REDUCTION OVER 48 HOURS
(% OF INITIAL)
100%
80%
60%
40%
20%
0%
*
†
NORMAL +VEH
NORMAL +DEX
ASTHMA +VEH
ASTHMA +DEX
*P=0.02
†P=0.04

FIG. 4B

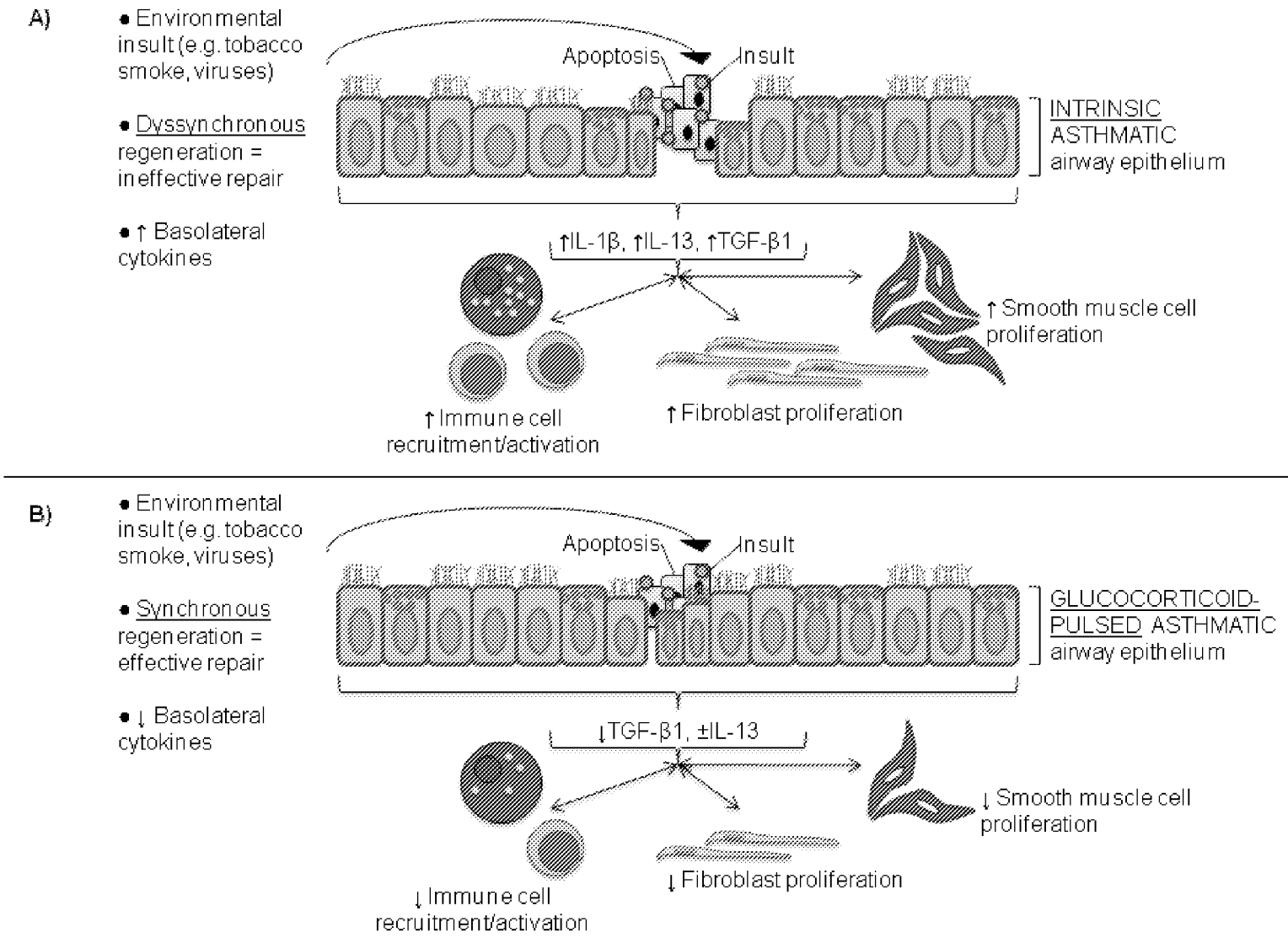
A)
• Environmental insult (e.g. tobacco smoke, viruses)
• Dyssynchronous regeneration = ineffective repair
• ↑ Basolateral cytokines
Apoptosis
Insult
INTRINSIC ASTHMATIC airway epithelium
↑IL-1β, ↑IL-13, ↑TGF-β1
↑ Smooth muscle cell proliferation
↑ Immune cell recruitment/activation
↑ Fibroblast proliferation
B)
• Environmental insult (e.g. tobacco smoke, viruses)
• Synchronous regeneration = effective repair
• ↓ Basolateral cytokines
Apoptosis
Insult
GLUCOCORTICOID-PULSED ASTHMATIC airway epithelium
↓TGF-β1, ±IL-13
↓ Smooth muscle cell proliferation
↓ Immune cell recruitment/activation
↓ Fibroblast proliferation

FIGURE 5

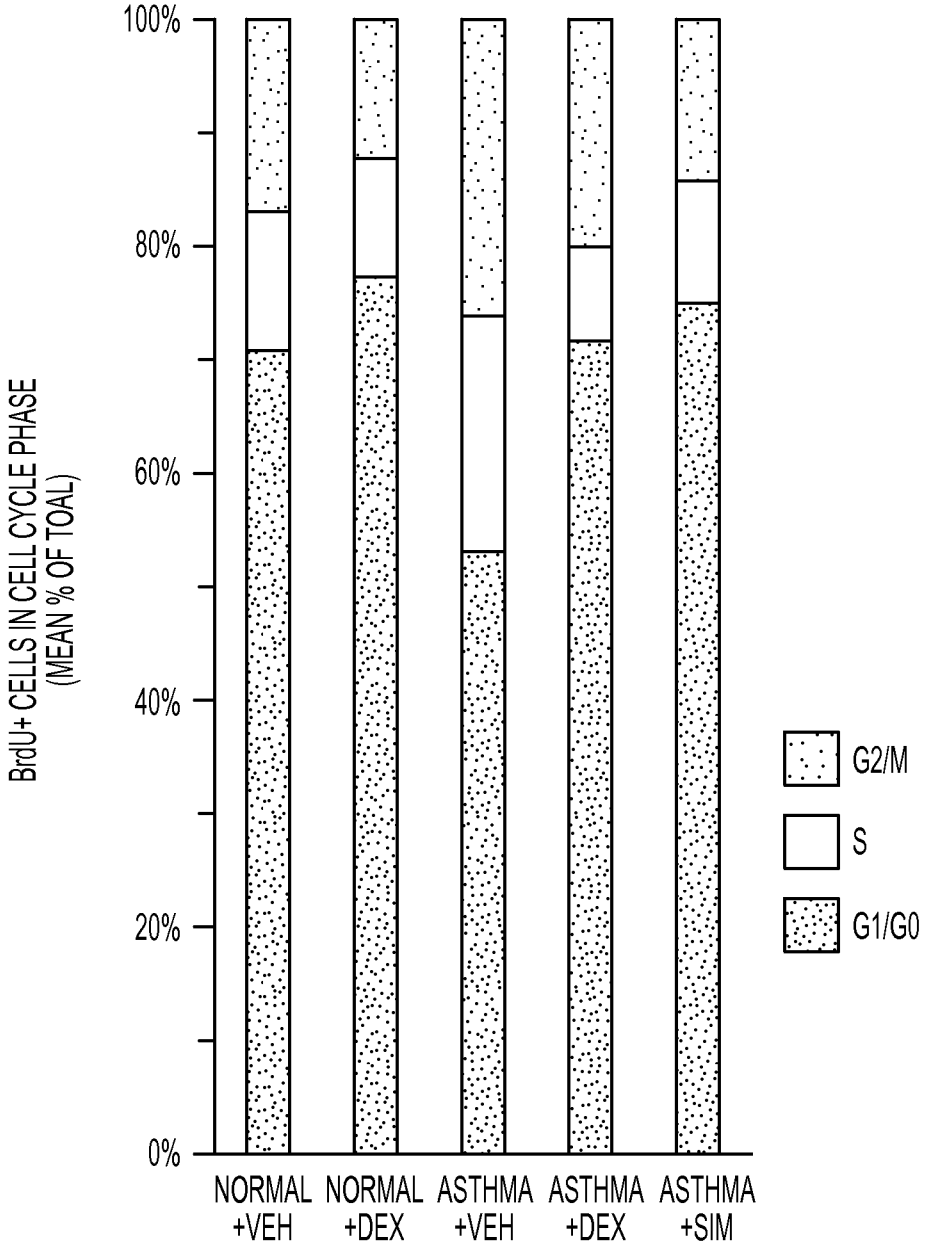
100%
80%
60%
40%
20%
0%
BrdU+ CELLS IN CELL CYCLE PHASE
(MEAN % OF TOAL)
NORMAL +VEH
NORMAL +DEX
ASTHMA +VEH
ASTHMA +DEX
ASTHMA +SIM
G2/M
S
G1/G0

FIG. 6

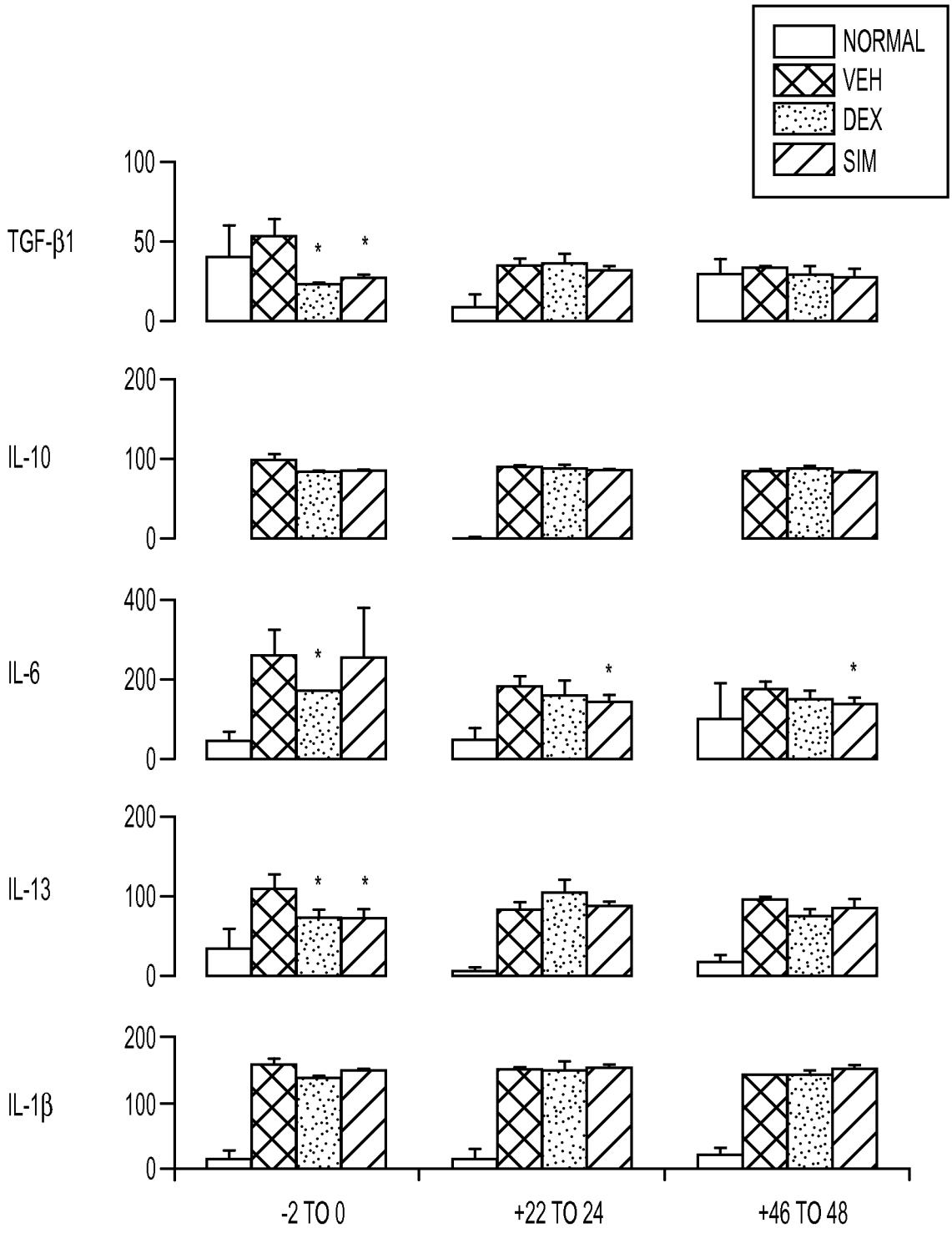
NORMAL
VEH
DEX
SIM
TGF-β1
IL-10
IL-6
IL-13
IL-1β
100
50
0
200
100
0
400
200
0
200
100
0
200
100
0
*
-2 TO 0
+22 TO 24
+46 TO 48

FIG. 7

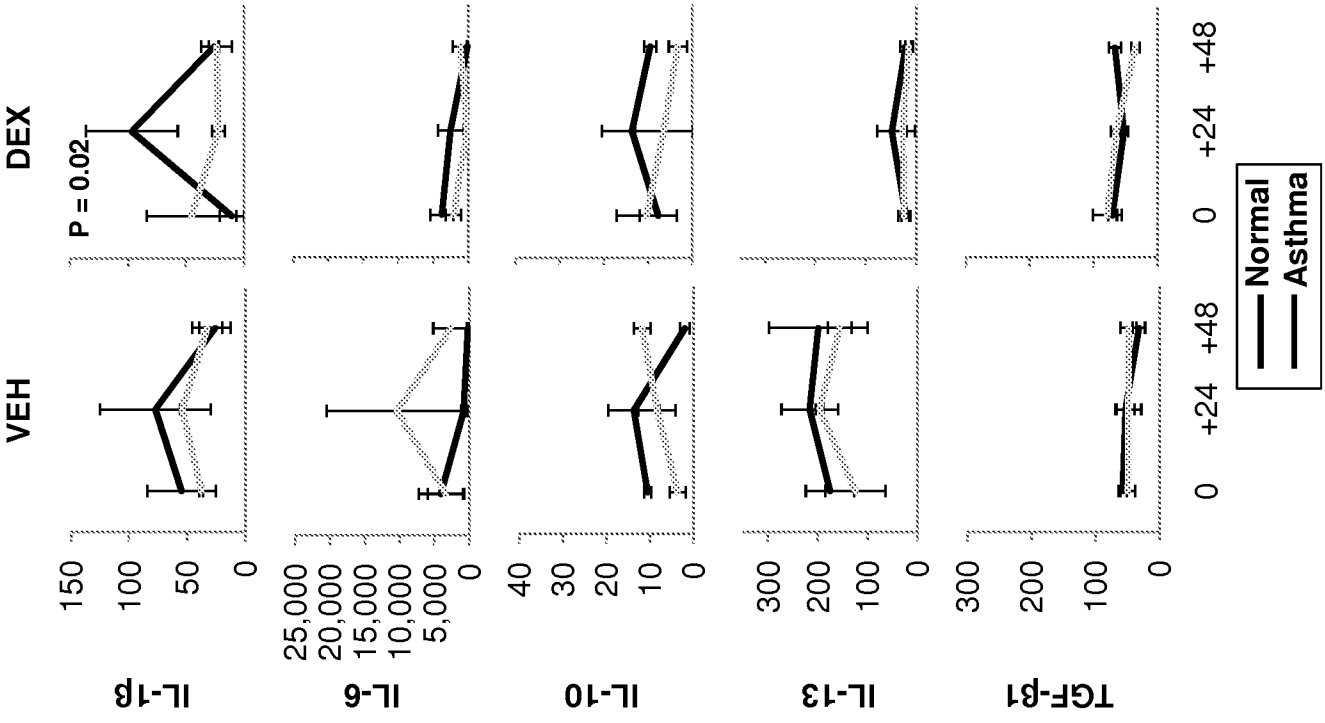
VEH
DEX
P = 0.02
IL-1β
IL-6
IL-10
IL-13
TGF-β1
150
100
50
0
25,000
20,000
15,000
10,000
5,000
40
30
20
10
300
200
0
+24
+48
Normal
Asthma

FIGURE 8