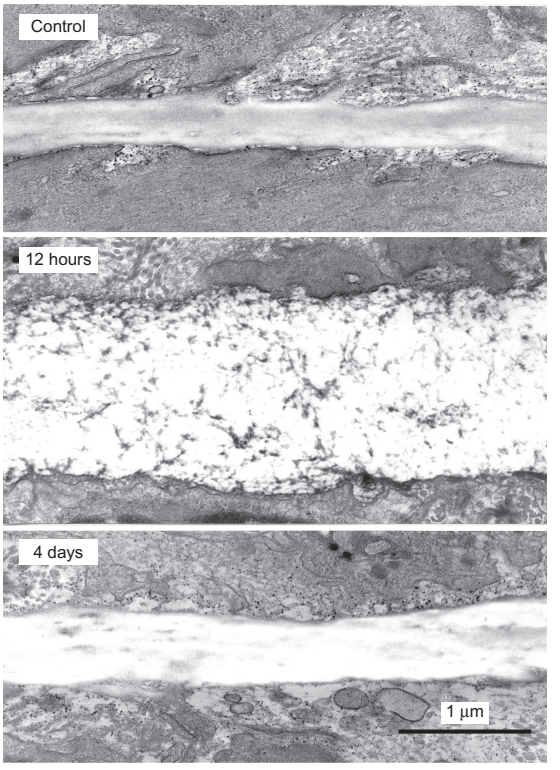
16

PULMONARY REGENERATIVE ENGINEERING



Influence of hypoxic pulmonary hypertension on the structure of the medial elastic laminae of the pulmonary arteries in the rat. Pulmonary arterial hypertension was induced by exposing rats to 10% oxygen and 90% nitrogen. The structure of the pulmonary arteries was examined by electron microscopy at 0, 0.5, and 4 days of exposure to hypoxia. An increase in the pulmonary arterial blood pressure induced rapid swelling and disorganization of the pulmonary arterial elastic laminae within 12 h of exposure to hypoxia. These pathological changes were transient. The pulmonary arterial elastic laminae regained their physiological morphology and appearance after 4 days of exposure to hypoxia without further noticeable changes even under a continuous identical hypoxic condition. See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

ANATOMY AND PHYSIOLOGY OF THE RESPIRATORY SYSTEM

Pulmonary Structure [16.1]

The pulmonary or respiratory system consists of the rib cage, respiratory skeletal muscles, diaphragm, pleura, and lung. The rib cage is the wall of the thoracic cavity. Respiratory muscles are attached to the ribs and other thoracic bones such as the clavicle bone. The diaphragm is a muscular and connective tissue membrane and constitutes the inferior border of the thoracic cavity. The pleura is a membrane system that covers the external surface of the lung, known as the visceral pleura, and internal surface of the rib cage, known as the *parietal pleura*. The two pleural membranes are continuous, forming a narrow, closed pleural cavity between the exterior surface of the lung and the internal surface of the rib cage (Fig. 16.1). As we will see below, this closed cavity plays a critical role in air ventilation of the lung.

The lung is composed of the airway, alveolar, vascular, and lymphatic systems. The lung is divided into the left and right lung in the thoracic cavity. The primary functions of the lung are blood oxygenation and carbon dioxide removal via gas ventilation and exchange. The airways are a tubular system responsible for gas ventilation. The alveoli constitute a membrane system responsible for gas exchange, specifically, oxygen transport from the air to the blood and carbon dioxide transport from the blood to the air. The vascular system carries deoxygenated blood from the systemic veins and right heart to the lung and delivers oxygenated blood from the lung to the left heart and systemic arteries. The lymphatic system collects and removes excessive fluid from the lung parenchyma. The anatomy and functions of these systems are described as follows.

The Airway and Alveolar Systems. The airway system consists of the larynx and various generations of airway. The *larynx* is a cartilage structure located between the pharynx and trachea and its function is to keep the trachea (the main airway) closed during swallowing, thus preventing water and foods from entering the airways and lung. The larynx also contains the vocal cords, which are structures for sound generation. The airways are a tubular system, consisting of tree-like cylindrical structures of various diameters. The largest airway is the trachea, which is composed of three layers: the mucosal, cartilage/smooth muscle, and connective tissue layers. The mucosa is constituted with two types of columnar epithelial cells: goblet and ciliated epithelial cells. The goblet epithelial cells secret mucus

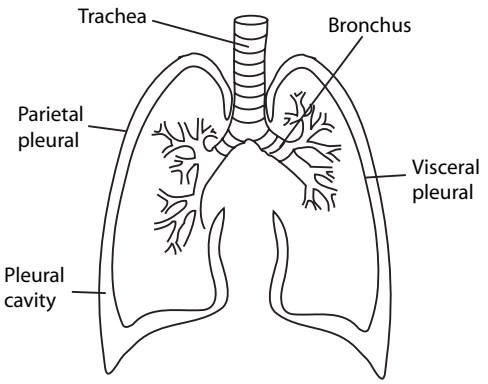


Figure 16.1. Schematic representation of the structure of the lung.

in response to the stimulation of inhaled particles. The ciliated epithelial cells are characterized by the presence of cilia at the cell surface. The cilia conduct periodic movements and are responsible for propelling and removing mucus and inhaled particles. In the middle layer, there is a series of C-shaped cartilage rings which alternate with connective/muscular tissue. The cartilage appears in the anterior and side wall, but not in the posterior wall of the trachea. The cartilage structures protect the trachea from collapsing.

The trachea is divided into the left and right bronchi, which enter the left and right lungs, respectively. The structure and cellular components of the left and right bronchi are similar to those of the trachea. Each bronchus is further divided into about 19 generations of bronchi with a graded decrease in diameter. The smallest airways are defined as bronchioles and the last generation is called terminal bronchioles. There are a large number of terminal bronchioles in the lung. The function of the bronchial tree is to conduct gases into and out of the lung. The structure of the small bronchi is different from that of the trachea and major bronchi. A major difference is that bronchi after the second generation do not contain cartilage rings. In addition, the density of goblet and ciliated epithelial cells reduces gradually as the airway diameter decreases. All bronchi contain smooth muscle cells, which control the diameter of the airways by contraction and relaxation.

The terminal bronchi are connected to the alveolar ducts, a structure mixed with tubular terminal bronchioles and alveoli. Alveoli are clusters of thin-walled, connected membrane sacs with an average size about 200 μm. There are about 300–400 million alveoli in the lung. The primary function of the alveoli is gas exchange between the blood and alveolar air. The wall of each alveolus is composed of a monolayer of alveolar epithelial cells on each side of the wall and a dense network of capillaries constituted with a monolayer of endothelial cells. There are two types of alveolar epithelial cells: type I and II epithelial cells. *Type I cells* are squamous thin epithelial cells that covers about 90% of the alveolar surface, where gas exchange takes places. *Type II cells* are specially differentiated epithelial cells which produce and secret surfactant, a mixture of lipids and proteins that spreads over the alveolar surface. The function of the surfactant is to reduce the surface tension of the alveolar wall at the interface between the air and the cell surface, ensuring even expansion and reduction of the alveoli through the entire lung during inspiration and expiration, respectively.

The Vascular System. The vascular system is composed of pulmonary arteries, capillaries, and veins. The pulmonary arteries originate from the right ventricle and are organized into a tree-like tubular system with a graded decrease in vessel diameter. Pulmonary arteries at each generation are arranged together with airways and pulmonary veins of the same generation. These arteries conduct deoxygenated blood from the right ventricle to the alveolar capillaries. A pulmonary capillary is a tube-like structure composed of a monolayer of endothelial cells about 6–10 μm in diameter and a basement membrane around the endothelial cells. The wall of alveoli contains a dense network of capillaries, where gas exchange occurs: oxygen diffuses from the alveolar air into the blood, and carbon dioxide diffuses from the blood to the alveolar air. The pulmonary veins are a tree-like tubular system, composed of multiple generations of veins and organized in parallel to the arterial system. The pulmonary veins conduct oxygenated blood from the capillaries to the left atrium.

The Lymphatic System. The pulmonary lymphatic system is a network of multiple generations of lymphatic vessels distributed around the airways and within the parenchymal tissue. The alveolar wall does not contain lymphatic vessels. The primary function of the lymphatic

system is to collect and remove excessive fluid from the parenchymal tissue. Because of the lack of lymphatic vessels at the alveolar level, the lung is susceptible to pulmonary edema, a disorder with excessive fluid in the parenchymal tissue and alveolar space. Pulmonary edema reduces the rate of gas exchange across the alveolar wall and is often found in patients with left heart failure, which induces an increase in blood pressure in the alveolar capillaries and excessive fluid transport from the blood to the alveolar space.

Pulmonary Function [16.1]

Gas Ventilation and Exchange. *Gas ventilation* is a cyclic process that includes an inspiration and an expiration phase. During the inspiration phase, fresh air is moved into the lung, whereas during the expiration phase exhaust air is removed from the lung. Gas ventilation is accomplished by coordinated action of a number of pulmonary constituents, including the airways, alveoli, pleural cavity, chest wall, skeletal muscles, and diaphragm. During the inspiration phase, the inward air flow is driven by a pressure gradient from the nasal cavity to the alveoli. The pressure gradient is generated by the contraction of inspiratory skeletal muscles, including the scalenes, pectoralis minor, external intercostals, and diaphragm. The contraction of these muscles increases the volume of the thoracic cavity. Because the pleural cavity is a closed negative system, the expansion of the thoracic cavity induces simultaneous expansion of the lung, resulting in a pressure gradient from the nasal cavity to the alveoli. During the expiration phase, the inspiratory muscles relax and the rib cage recoiled back, resulting in a decrease in the thoracic cavity and the volume of the lung. The volumetric change establishes an adverse pressure gradient from the alveoli to the nasal cavity, which removes the exhaust air from the lung.

Gas exchange is a process of gas diffusion across the alveolar epithelial and endothelial cells, involving oxygen and carbon dioxide. Oxygen diffuses from the alveolar air space into the blood, whereas carbon dioxide diffuses from the blood to the alveolar space. The driving force for gas diffusion is the gradient of partial gas pressure, which is defined as the pressure contributed by a specified type of gas in a mixture of multiple gases. The total air pressure is about 760 mm Hg at the sea level. The *oxygen partial pressure* (Po_2) is about 155 mm Hg in the air, about 105 mm Hg in the alveolar space, and about 40 mm Hg in the deoxygenated venous blood in the alveolar capillary network (Fig. 16.2). The difference of Po_2 is about 65 mm Hg across the alveolar wall. This partial pressure difference drives oxygen diffusion from the alveolar space to the blood. Since oxygen diffusion is very efficient across the alveolar wall and the capillaries are fairly long, the capillary blood can be saturated with oxygen within the first half of the capillary length. Oxygenated blood is conducted to the pulmonary veins, left heart, and the arterial system.

At the same time, carbon dioxide diffuses from the blood to the alveolar space based on a gradient of partial pressure. The *partial pressure of* CO_2 is about 45 mm Hg in the deoxygenated venous blood and about 40 mm Hg in the alveolar space. The difference of carbon dioxide partial pressure across the alveolar wall drives CO_2 diffusion from the blood to the alveolar space. During expiration, the exhaust air containing a higher concentration of CO_2 is removed from the lung.

Ratio of Air Ventilation to Blood Perfusion. The rate of air ventilation in a region of the lung or in the entire lung is proportional to the rate of blood perfusion within the same region. The ratio of air ventilation to blood perfusion is about 0.75 under physiological conditions. The pulmonary system intends to maintain this ratio within a narrow range around 0.75 under pathological conditions. For instance, when a bronchus is partially

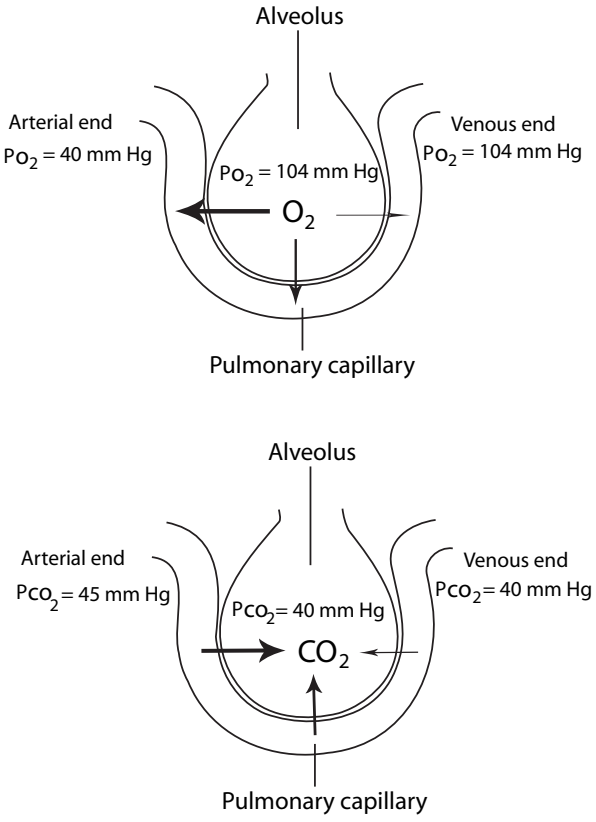


Figure 16.2. Schematic representation of the structure of the airways and alveoli. Based on bibliography 16.1.

blocked with a tumor, the rate of air ventilation is accordingly reduced in the alveolar system distal to the blocked bronchus, resulting in a reduction in the ventilation-to-perfusion ratio. Reduced oxygen or hypoxia in the under-ventilated region enhances the contractility of the arterial smooth muscle cells and induces arterial constriction, reducing the rate of blood perfusion into the region. Such a response brings up the ventilation-to-perfusion ratio toward the physiological level. With such a mechanism, the ventilation-to-perfusion ratio is maintained at a relatively constant range. The physiological significance of such a mechanism is to redistribute blood volume to well-ventilated regions and to ensure a sufficient volume of fully oxygenated blood.

Control of Gas Ventilation. There are two types of respiratory movement: involuntary and voluntary. The *involuntary movement* is rhythmic and responsible for the maintenance of the basal level of air ventilation. Such a movement is controlled by the respiratory center located in the medulla oblongata of the brainstem. In the case of increased physical exercise, which demands more oxygen in the skeletal muscle system and the heart, the respiratory center increases the contraction frequency and depth of the respiratory muscles, resulting in an increase in air ventilation and oxygen supply to the alveoli. At the same time, the heart beating rate and cardiac contractility are also increased under the influence of activated cardiovascular control center, resulting in an increase in blood supply to the lung. The increased blood supply can be fully oxygenated because of enhanced air

ventilation. The *voluntary movement* causes forced respiration. Such an activity is controlled by the cortex neurons for regulating the respiratory muscles. Under physiological conditions, it is not necessary to carry out voluntary respiration.

PULMONARY DISORDERS

Asthma

Pathogenesis, Pathology, and Clinical Features [16.2]. *Asthma* is defined as a chronic disorder of the airways characterized by increased responsiveness of the airways to a multiplicity of stimuli and inflammatory reactions in the airway wall. It is manifested by widespread narrowing of the air passages in association with clinical symptoms, such as paroxysms of dyspnea, cough, and wheezing. The clinical symptoms may be relieved spontaneously or as a result of therapy. Asthma is an episodic disease with acute exacerbations interspersed with symptom-free periods. Typically, most attacks are short-lived, lasting minutes to hours. The patient can recover completely after an attack. However, there can be a phase in which the patient experiences some degree of airway obstruction daily. This phase can be mild, with or without superimposed severe episodes, or much more serious, with severe obstruction persisting for days or weeks. The latter condition is known as *status asthmaticus*. In unusual circumstances, acute episodes can cause death.

Asthma is a chronic airway disorder characterized by transient airway constriction or obstruction (Fig. 16.3), airway inflammation and remodeling (Fig. 16.4), airway cell

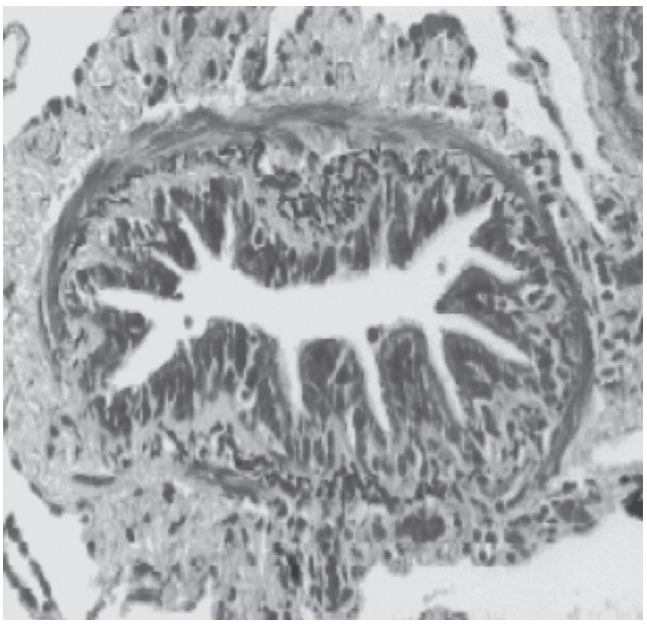


Figure 16.3. Airway remodeling in asthma. Remodeling of small airway in chronic asthma. Note connective tissue deposition in subepithelial and adventitial compartments of the airway wall. Connective tissue deposition seems to encase the airway smooth muscle bundles. (Reprinted from Chung KF: The role of airway smooth muscle in the pathogenesis of airway wall remodeling in chronic obstructive pulmonary disease, *Proc Am Thorac Soc*) 2:347–54, copyright 2005, with permission from American Thoracic Society.)

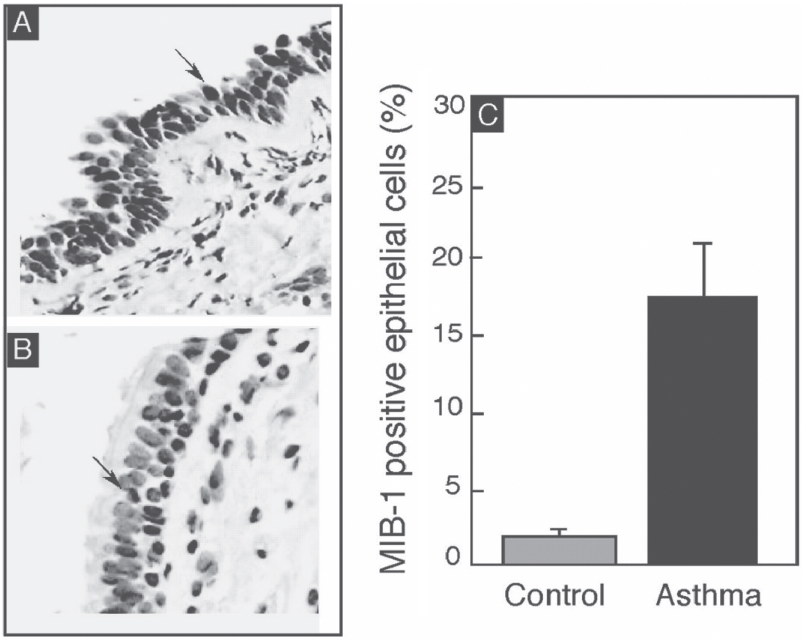


Figure 16.4. Airway cell proliferation in control and asthma. Cell proliferation was detected by an antibody (MIB1) directed against the Ki-67 antigen, a marker for proliferating cells. The black color indicates positive MIB1 staining in the asthmatic epithelial cells (A) and healthy controls (B). (C) The graph shows MIB1-positive cells (mean ± SD) of three healthy controls and four asthmatics. Note that some fields in asthmatic airways show more than 80% MIB1-positive cells. Arrows show positive cells. (Reprinted with permission from Comhair SA et al: Superoxide dismutase inactivation in pathophysiology of asthmatic airway remodeling and reactivity, *Am J Pathol* 166:663–74, copyright 2005.)

proliferation (Fig. 16.5), and persistently increased responsiveness and contractility of airway smooth muscle cells in response to the stimulation of allergens, environmental factors, and pharmacological agents. Typical asthmatic allergens include air-borne particles derived from plants (e.g., grass pollens, ragweed pollens, birch pollens, mountain cedar pollens, and peanuts), animals (cat and dog fur dusts), and microorganisms (bacteria and viruses). Examples of environmental factors include air pollutants, chemicals, metal particles, dusts, and polymer particles. Pharmacological agents that cause asthma include antibiotics, aspirin, and β-adrenergic antagonists. In addition, viral infection of the lung, physical exercise, and emotional stress can initiate asthmatic attacks. All these factors can cause an increase in the reactivity of the airway smooth muscle cells, resulting in airway constriction and a reduction in the rate of ventilation. Asthmatic attacks are usually transient, lasting for a period from minutes to hours. The clinical consequence of asthma is dependent on the degree of airway constriction and oxygen deficiency. Severe airway constriction is often life threatening. Asthma occurs frequently in children and young adults. Asthmatic patients often have a family history of allergic diseases.

The pathogenic mechanisms of asthma vary, depending on the factors that cause asthma. Allergic asthma, one of the most common types, is related to enhanced immune

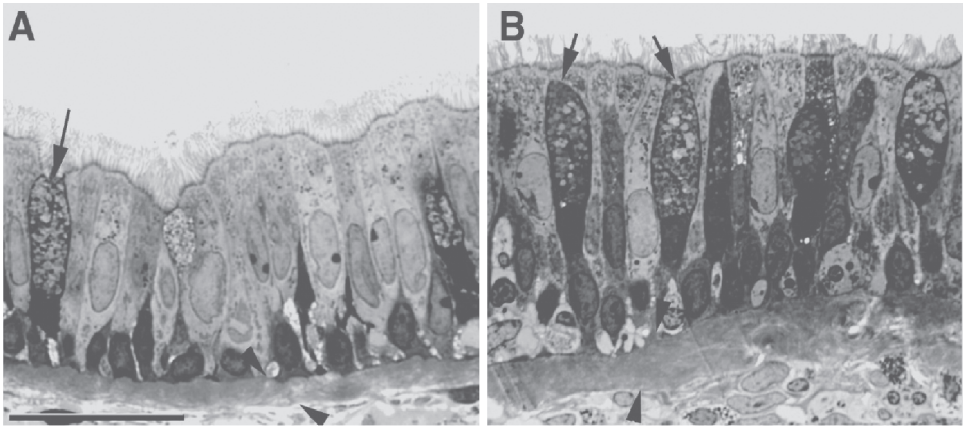


Figure 16.5. Inflammatory changes in asthmatic airways in rhesus monkeys. Histopathological comparison of epithelial morphology in the intrapulmonary bronchi of sensitized (B) and nonsensitized (A) rhesus monkeys with house dust mite (*Dermatophagoides farinae*). Note that the asthmatic airway was associated with inflammatory cell infiltration, epithelial hypertrophy, and mucous goblet cell hyperplasia (arrows). Scale bar: 20 μm. (Reprinted with permission from Schelegle ES et al: Allergic asthma induced in rhesus monkeys by house dust mite (Dermatophagoides farinae), *Am J Pathol* 158:333–41, copyright 2001.)

responses of lymphocytes exposed to allergens. In patients genetically susceptible to asthma, a primary exposure to an allergen in the airway system leads to the activation of the antigen-presenting dentritic cells (Fig. 16.6). These cells present the inhaled antigen to $CD4^+$ T-helper cell precursors in the bronchial lymph nodes, potentially inducing the differentiation of the precursor cells into two types of cells: type 1 and type 2 T-helper cells. The fate of the differentiation is dependent on the presence of dominant cytokines. When interleukin (IL)12 is dominant, the precursor cells are induced to differentiate into type 1 T-helper cells. In contrast, in the presence of dominant IL4, the precursor cells are differentiated into type 2 T-helper cells. The type 1 T-helper cells can produce interferon-β, IL2, and Tumor necrosis factor (TNF)α. These factors exert inhibitory effects on asthmatic activities. In contrast, the type 2 T-helper cells produce IL4, IL5, and IL9, which promote asthmatic activities.

The type 2 T-helper cell cytokines can stimulate B lymphocytes to produce IgE antibodies against the allergen. These antibodies can attach to the surface of mast cells. A secondary exposure to the same allergen induces reaction of the allergen with the IgE antibodies attached to the mast cells, resulting in antibody activation. The activated antibodies in turn stimulate mast cells to release inflammatory factors, such as histamine, prostaglandins, and bradykinin, causing the contraction of airway smooth muscle cells. These inflammatory factors also cause mucosal edema, an increase in mucus secretion, and infiltration of leukocytes, especially eosinophils. IgE and allergens can bind to eosinophils, inducing the release of major basic protein (MBP) from the eosinophils. MBP can cause airway injury and inflammation. All these pathological changes contribute to the reduction in the luminal area of the bronchi. As asthma attacks continue, chronic inflammatory reactions may occur, inducing persistent leukocyte infiltration, mucosal thickening, and airway constriction.

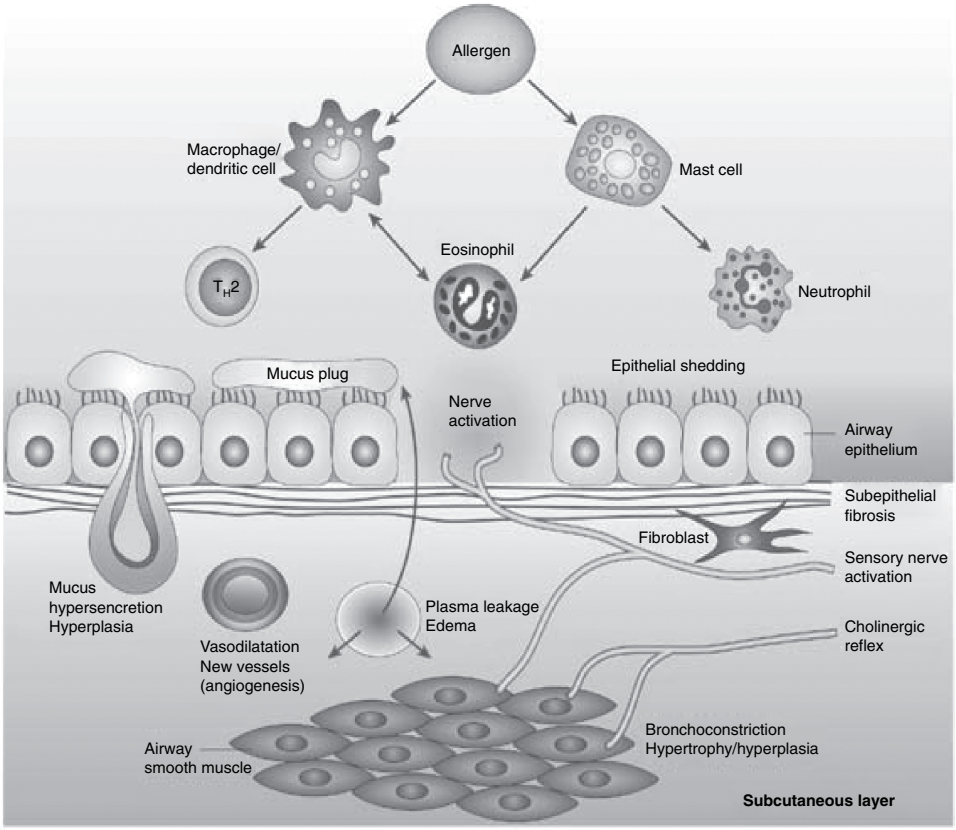


Figure 16.6. Pathogenesis of asthma. Several inflammatory cell types=s are recruited to and/or activated in the airways, releasing a variety of inflammatory mediators that have acute effects on the airway, such as bronchoconstriction, plasma leakage, vasodilatation, mucus secretion, sensory nerve activation, and cholinergic reflex-induced bronchoconstriction. These acute changes are followed with structural remodeling, resulting in subepithelial fibrosis, increased numbers of blood vessels and mucus-secreting cells, and increased thickness of airway smooth muscle, and airway hyperplasia and hypertrophy. (Reprinted by permission from Macmillan Publishers Ltd.: Barnes PJ: New drugs for asthma, *Nature Revs Drug Discov* 3:831–44, copyright 2004.)

Asthma is associated with several pathological changes. At the cellular and tissue level, there often exist epithelial cell detachment, airway mucosal edema, eosinophil infiltration, subepithelial fibrosis, basal lamina thickening, and smooth muscle hypertrophy and hyperplasia (Fig. 16.4). In addition, goblet cell hyperplasia occurs (Fig. 16.5), inducing mucus overproduction and secretion. All these changes contribute to the reduction in the airway diameter. In asthma, the sensory nerve endings are sensitized to a certain extent, contributing to the elevation of the smooth muscle tone. In severe cases, an apparent change is lung overexpansion due to airway obstruction and air retention in the alveoli.

Experimental models of asthma [16.3]. Experimental asthma can be induced by sensitizing rats or mice by administration of allergens. A common allergen used in asthma

induction is ovalbumin. For primary allergen sensitization, a mixture of ovalbumin (10–100 μg) and aluminum hydroxide 1 mg in 0.2–1 ml saline can be prepared and injected into the peritoneal cavity of an animal three times at day 0, 7, and 14. The animal can be rechallenged by exposure to ovalbumin aerosol later. The presence of asthma can be assessed by measuring pathological changes, such as airway mucosal edema, smooth muscle hypertrophy, epithelial cell detachment, eosinophil infiltration, mucus overproduction, basal lamina thickening, and airway constriction.

Conventional Treatment of Asthma [16.4]. Several approaches have been developed and used to treat asthma, including the removal of the causative factors, immunotherapy, and drug therapy. The removal of the causative factors is an effective approach for the prevention of asthma. However, it is usually difficult to find the cause of asthma. Even though the causative factors are known, it is impossible to remove them completely from the environment.

Allergen immunotherapy is an effective method for the treatment of asthma. The hypothetical basis for immunotherapy is that the introduction of a selected allergen via a parenteral route may activate regulatory T lymphocytes, which inhibit Th2 lymphocytes and thus reduces the production of IgE antibodies by B lymphocytes. These changes lead to reduced activity of the immune system to subsequently inhaled allergens. To carry out immunotherapy, allergens specific to a patient should be identified, prepared, and used for therapeutic purposes.

Several types of drugs have been used to treat asthma. These include bronchodilators and glucocorticoids. Bronchodilators include β-adrenergic agonists and anticholinergic agents. β-adrenergic agonists, such as epinephrine, isoproterenol, and resorcinols, activate the β-adrenergic receptor of the airway smooth muscle cells, inducing airway dilation and relieving the symptoms of asthma. Anticholinergic agents, such as atropine sulfate, atropine methylnitrate, and ipratropium bromide, can be used to suppress acetylcholine, a substance that stimulates airway smooth muscle contraction, and thus to induce airway dilation. Airway inhalation is an effective method for the delivery of these agents. However, most bronchodilators stimulate cardiac activities and should be used with caution for patients with cardiac diseases. Glucocorticoids are hormones produced in the cortex of the adrenal gland and can be used to suppress inflammatory reactions. Since inflammation occurs in asthma and contributes to the obstruction of bronchi, glucocorticoids are often used to reduce inflammation.

Molecular Therapies for Asthma [16.5]. Based on the pathogenic mechanisms of asthma, two molecular therapeutic strategies have been developed: (1) suppressing inflammatory reactions and mucus secretion, and (2) inducing airway dilation. The suppression of airway inflammation can be achieved by the transfection of target cells with antiinflammatory cytokine genes and the glucocorticoid receptor gene, administration with inhibitors to inflammatory transcription factors and kinases, and the blockade of inflammatory cytokines and cytokine receptors (Fig. 16.7). Airway dilation can be achieved by transferring the β_2-adrenergic receptor gene. These approaches are described as follows.

Suppression of Asthmatic Changes by Administration of Antiinflammatory Cytokine Genes, Antibodies, and Inhibitors [16.6]. There are several approaches for inhibiting allergic responses and asthmatic changes. These include the transfection of target cells with antiinflammatory cytokines or their genes, administration with antibodies against

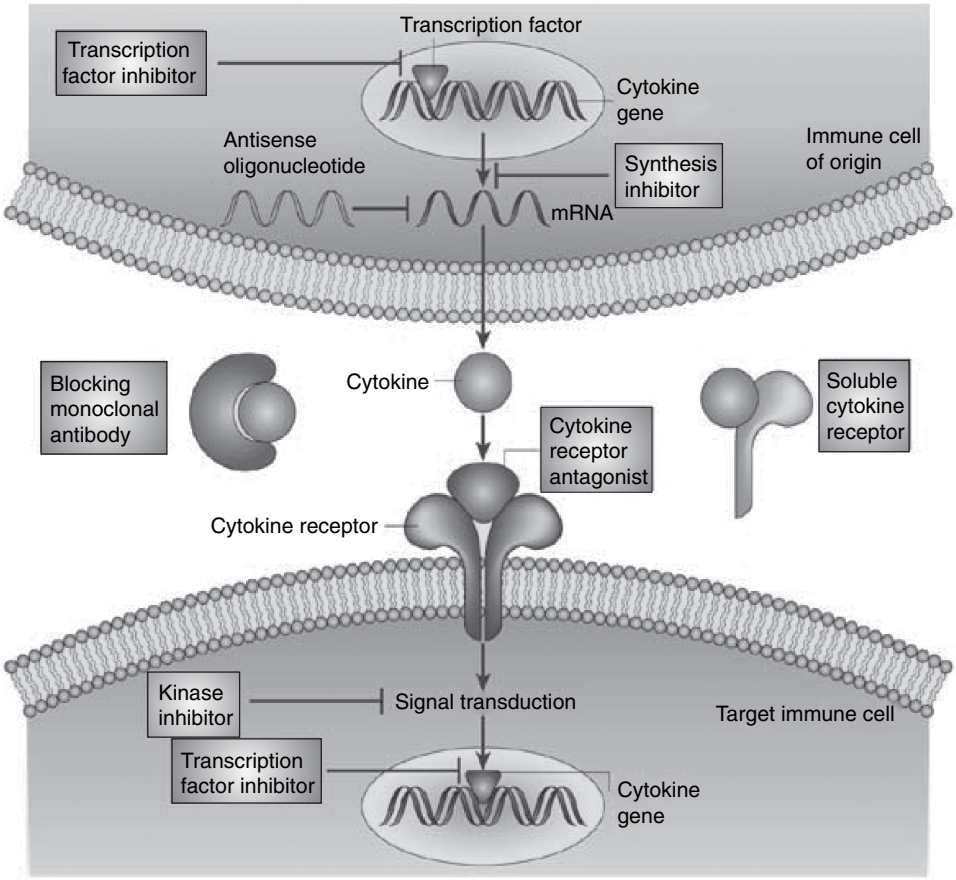


Figure 16.7. Strategies for inhibiting proinflammatory cytokines in asthma. These include inhibition of cytokine synthesis (e.g., corticosteroids), inhibition of transcription factors regulating cytokine expression (e.g., calcineurin inhibitors or decoy oligonucleotides), inhibition of secreted cytokines with blocking antibodies [e.g., antiinterleukin (IL)5 antibody] or soluble receptors (e.g., soluble IL4 receptors), blocking cytokine receptors (e.g., chemokine receptor antagonists), blocking signal-transduction pathways (e.g., p38 mitogen-activated protein kinase inhibitors) or transcription factors activated by cytokines (e.g., STAT6 inhibitors). (Reprinted by permission from Macmillan Publishers Ltd.: Barnes PJ: New drugs for asthma, *Nature Revs Drug Discov* 3:831–44, copyright 2004.)

inflammatory factors, and administration with inflammation inhibitors (Fig. 16.8). There are a number of antiinflammatory cytokines, including interleukin (IL)10, interleukin-12, and interferon-γ. Interleukin-10 is a cytokine that suppresses inflammatory reactions in the airway. In patients with asthma, the expression of the interleukin-10 gene is reduced compared to the normal population. In experimental models, the administration of interleukin-10 significantly suppresses inflammatory reactions in response to the stimulation of allergens. Immunotherapeutic approaches often induce upregulation of the interleukin-10 gene. These observations suggest that interleukin-10 or its gene can be used as therapeutic agents for the treatment of asthma.

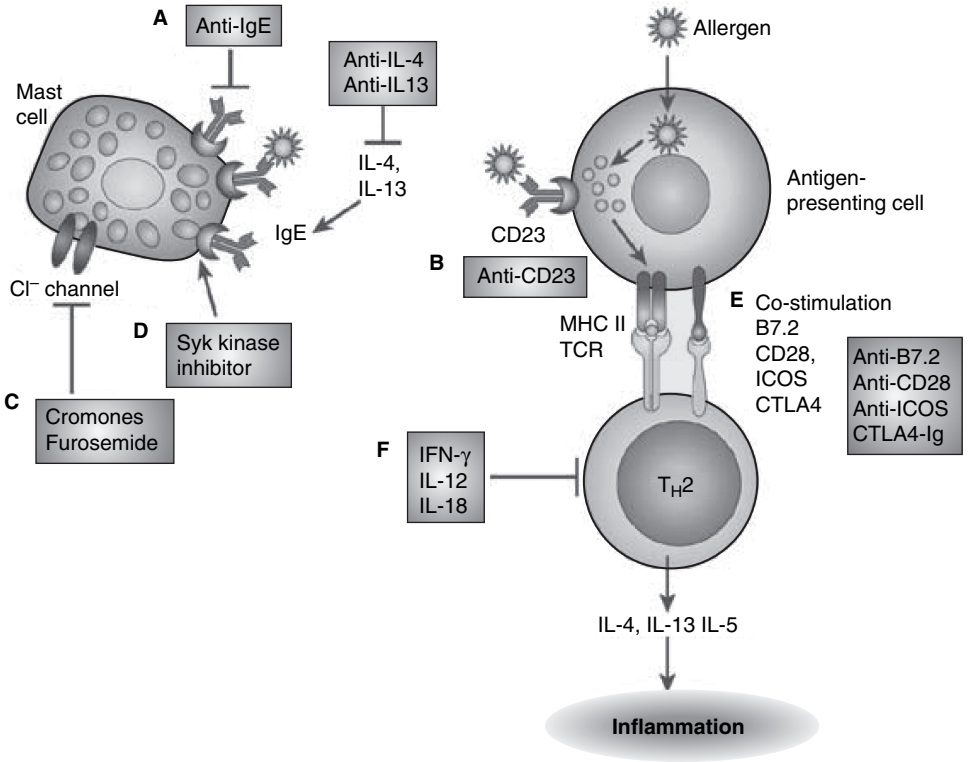


Figure 16.8. Strategies for inhibiting allergic responses underlying asthma. Immunoglobulin E (IgE) can be inhibited by the antibody omalizumab (A) and low-affinity IgE receptors by anti-CD23 (B). Mast cells can also be blocked by cromones and furosemide (C), probably acting on a chloride channel and by inhibitors of Syk kinase, which inhibit the signal-transduction pathways activated by IgE receptors (D). Antigen presentation can be blocked by inhibitors of costimulatory molecules (E), including B7.2, CD28, inducible costimulatory molecule (ICOS), and cytotoxic T-lymphocyte antigen-4 (CTLA4). T_H2 cells can also be directly inhibited by interferon-γ (IFNγ), interleukin (IL)12 and IL18 (F). (Reprinted by permission from Macmillan Publishers Ltd.: Barnes PJ: New drugs for asthma, *Nature Revs Drug Discov* 3:831–44, copyright 2004.)

IL12 is a cytokine that suppresses the production of asthma-promoting cytokines from the type 2 T-helper cells, thus exerting an inhibitory effect on asthmatic activities, such as mucus secretion, inflammation, and eosinophil infiltration. Experimental investigations have demonstrated that the transfer of the IL12 gene into the lung of ovalbumin- or dust mite-sensitized animals results in an increase in the level of interferon-γ, an anti-asthmatic cytokine, and a reduction in the activity of the type 2 T-helper cells, eosinophil infiltration, and inflammatory reactions. The cotransfer of IL12 with IL18, another anti-asthmatic cytokine, elicits a synergistic inhibitory effect on asthmatic changes. The IL12 and IL18 genes are potential candidates for the treatment of human asthma. Furthermore, the activation of nitric oxide synthase type 2 is required for the activity of IL12. Thus a cotransfer of the nitric oxide synthase type 2 gene with the IL12 gene may enhance the anti-asthmatic effect of IL12. The characteristics of IL12 and IL18 are presented in the following table.

Interferon-γ is a cytokine that suppresses inflammatory reactions and inhibits the production and secretion of asthma-promoting cytokines, including IL4 and IL5 (note that these cytokines stimulate the activation and migration of eosinophils and secretion of inflammatory agents such as IgE immunoglobulins), from the type 2 T-helper cells, and thus reduce asthmatic activities (see Table 16.1 for characteristics of interferon-γ). In several experimental investigations, interferon-γ gene has been transferred into the lung of asthmatic animals by using virus-mediated gene transfer approaches. These investigations have demonstrated that the overexpression of the interferon-γ gene is associated with a reduction in the density of eosinophils and mucus secretion, resulting in a decrease in asthmatic symptoms. Clinical investigations have demonstrated similar results. Interferon-γ and its gene have been proven effective antiinflammatory molecules that can be used for the treatment of asthma.

In addition to the administration of antiinflammatory cytokines and their genes, the inflammatory cytokines can be suppressed by local delivery of antisense oligonucleotides or small interfering RNA specific to these cytokines. As discussed on pages 448 and 449, antisense oligonucleotides or small interfering RNA (siRNA) are short nucleotide sequences, can be synthesized based on the sequence of target mRNA, and can be used as therapeutic agents. A selected agent can be delivered via inhalation to the airways. These short nucleotide sequences can be taken up by airway epithelial cells. Experimental investigations have demonstrated that either oligonucleotides or small siRNA can effectively suppress inflammatory reactions in the airways. Other potential therapeutic approaches are presented in Fig. 16.8.

Characteristics of asthma-related interleukins are listed in Table 16.1.

Suppression of Inflammatory Reactions by Transferring the Glucocorticoid Receptor Gene [16.7]. Glucocorticoid is a hormone produced in the cortex of the adrenal glands. It interacts with the glucocorticoid receptor and suppresses inflammatory reactions, such as leukocyte infiltration, the production of asthmatic cytokines, and mucus secretion. The glucocorticoid receptor, also known as GCR and nuclear receptor subfamily 3 group C member 1 (NR3C1), is a nuclear receptor of 777 amino acids and about 86 kDa. This receptor is expressed in a variety of cell and tissue types, including leukocytes, cardiomyocytes, brain, lung, kidney, skeletal muscle, liver, pancreas, intestine, eye, skin, and osteoblasts. The primary functions of this receptor are to interact with glucocorticoid and act as a transcription factor to induce the expression of antiinflammatory genes. Thus the activation of glucocorticoid receptor results in the suppression of inflammatory reactions. In vitro investigations by using human airway cells have demonstrated that the overexpression of the glucocorticoid receptor gene results in the suppression of several transcriptional factors, such as activator protein 1 and nuclear factor κB, which induce inflammatory reactions. Further investigations are necessary to verify these antiinflammatory activities in vivo.

Inducing Bronchodilation by Transferring Bronchodilator Genes and Proteins [16.8]. There are several types of bronchodilator proteins, including the β_2-adrenergic receptor and atrial natriuretic peptide (ANP). The β_2-adrenergic receptor of the airway smooth muscle cells interacts with epinephrine and induces smooth muscle relaxation and airway dilation, an effective approach for the relief of asthmatic symptoms. The transfection of the β_2-adrenergic receptor gene into the airway smooth muscle cells can up-regulate

TABLE 16.1. Characteristics of Selected Asthma-Related Interleukins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
IL12α	IL12A, IL-12A, interleukin 12 α chain, IL12 subunit p35, cytotoxic lymphocyte maturation factor (CLMF), cytotoxic lymphocyte maturation factor 35-kDa subunit (CLMF p35), natural killer cell stimulatory factor 35-kDa subunit (NKSF1), NK cell stimulatory factor chain 1, T-cell-stimulating factor (TSF)	219	25	Dendritic cells	Stimulating T-cell-independen induction of interferon-γ, inducing the differentiation of Th1 cells, suppressing the production of asthma-promoting cytokines from the Th2 cells, and sustaining memory/ effector Th1 cells to mediate long-term immune protection
IL12β	IL12B, IL12 subunit p40, interleukin 12 β chain, cytotoxic lymphocyte maturation factor 40-kDa subunit (CLMF p40), NK cell stimulatory factor chain 2 (NKSF2), IL23 subunit p40, cytotoxic lymphocyte maturation factor 2 (CLMF2), natural killer cell stimulatory factor 40-kDa subunit, T-cell-stimulating factor (TSF)	328	37	Dendritic cells, B cells, macrophages	Forming heterodimer via disulfide bonds
IL13	P600	132	14	Th2 cells, skin	Regulating B- cell maturation and differentiation, promoting IgE isotype development of B cells, and promoting asthmatic activities

*Based on bibliography 16.6.

**Note that other asthma-related interleukins and interferons are listed on page 631.

the expression of the receptor and enhance the activity of the bronchodilator epinephrine. Experimental investigations have demonstrated the effectiveness of this approach for the treatment of asthma. Atrial natriuretic peptide (ANP) serves as a potent bronchodilator. In experimental investigations, this peptide can protect the airways from chemical-induced bronchoconstriction. Blood delivery and local application of atrial natriuretic peptide are effective therapeutic approaches for the treatment of asthma.

Cystic Fibrosis

Pathogenesis, Pathology, and Clinical Features [16.9]. *Cystic fibrosis* is a fatal, autosomal, inherited disease which involves multiple systems, including the pulmonary, cardiovascular, gastrointestinal, skeletal, and reproductive systems. Cystic fibrosis in the pulmonary system is characterized by the presence of profound inflammation, bronchitis, bronchopneumonia, lung abscesses, atelectasis, pulmonary hypertension, cor pulmonale, and congestive heart failure. The pulmonary involvement is a major cause of death in cystic fibrosis. Cystic fibrosis is a common disease in the Caucasian population and the occurrence is about 1 in 2500. This disorder is often found in children.

The pathogenesis of cystic fibrosis is related to the mutation of a gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR), also known as cystic fibrosis transmembrane conductance regulator ATP binding cassette, MRP7, ABC35, and ABCC7 (1480 amino acids and 169 kDa). CFTR is a member of the superfamily of ATP-binding cassette (ABC) transporters and is found in the epithelial membrane of the lung and intestine. It serves as a cAMP-regulated chloride channel. CFTR mediates the transport of chloride as well as other ions such as sodium and HCO_3^- (Fig. 16.9). The mutation of the CFTR gene induces the disorder of ion transport across the epithelial cells, resulting in a reduction in chloride secretion and an increase in sodium absorption. These changes influence the function of epithelial cells and contribute to the development of cystic fibrosis. There are several types of genetic alterations, which may influence the function of the CFTR protein: null expression, mutation, and regulatory disorder of CFTR. CFTR gene mutation is the most common cause of cystic fibrosis. A typical mutation is the deletion of the 508 phenylalanine. In the lung of cystic fibrosis, the concentration of interleukin-10, an antiinflammatory cytokine, is reduced compared to that in normal people. This change also contributes to inflammatory reactions in cystic fibrosis.

In cystic fibrosis, pathological examinations often exhibit excessive growth of bronchial goblet cells and increased secretion of mucus. Inflammatory reactions are found around bronchi with increased leukocyte infiltration. These changes usually lead to partial or complete obstruction of the bronchi. Patients with cystic fibrosis are often associated with infection by bacteria, such as *Pseudomonas aeruginosa* and *E. coli,* viruses, and fungi. It is often difficult to remove these micro-organisms from the airways because of the functional impairment of the ciliated epithelial cells. As the disorder develops, extensive fibrosis in the parenchyma and bronchi occurs (Fig. 16.10), which is associated with irreversible bronchial distortion and obstruction, increased stiffness of parenchyma, and reduced ventilation. Fibrous disorders also occur in the alveolar wall, resulting in the impairment of oxygen exchange. Patients usually die of oxygen deficiency due to impaired gas ventilation and exchange.

Experimental Models of Cystic Fibrosis [16.10]. Experimental cystic fibrosis can be induced by introducing *P. aeruginosa* into the airways of the rat, mouse, or other animals.

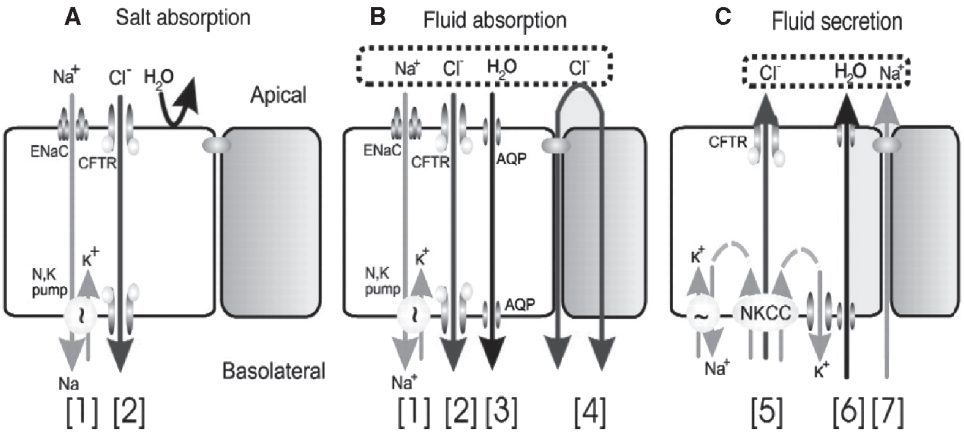


Figure 16.9. CFTR's multiple roles in fluid and electrolyte transport. (A) Salt absorption. In the sweat duct, high apical conductance for Na^+ [1] and Cl^- [2] and relatively low water conductance allows salt to be reabsorbed in excess of water (hypertonic absorption), leaving a hypotonic luminal fluid. In the sweat duct CFTR is the only available anion conductance pathway, and when it is lost in CF the lumen quickly becomes highly electronegative and transport virtually ceases, resulting in high (similar to plasma) luminal salt (B). Fluid absorption. In epithelia with high water permeability (3) relative to electrolyte permeability water will absorbed osmotically with salt to decrease the volume of luminal fluid. If no other osmolytes or forces are present, the salt concentration will remain unchanged. If water-retaining forces are present, permeant electrolytes can be reduced preferentially. The consequences of eliminating CFTR depend on the magnitude of such forces, the relative magnitude of alternate pathways for transepithelial anion flow (4), and how CFTR affects other ion channels. The high-salt and low-volume hypotheses differ on each of these points. (C) Anion-mediated fluid secretion. Secreting epithelia lack a significant apical Na^+ conductance. Basolateral transporters such as NKCC move Cl^- uphill into the cell; it then flows passively into the lumen via CFTR [5], K^+ exits basolaterally, Na^+ flows paracellularly and water follows transcellularly. Elimination of CFTR eliminates secretion. (Reprinted with permission from JJ: Wine The genesis of cystic fibrosis lung disease, *J Clin Invest* 103:309–12, 1999.)

Agarose beads can be used as carriers for bacterial delivery. To prepare *P. aeruginosa*-containing agarose beads, *P. aeruginosa* can be isolated from the airway of human cystic fibrosis patients, mixed with an agarose solution, and grown to near saturation. The bacterial and agarose solution is added to a bath of mineral oil at temperature 50ºC, stirred for several minutes, and placed on ice for several minutes. Agarose beads form in the oil bath when temperature reduces. The agarose beads can be washed in 0.5% deoxycholic acid in phosphate buffered saline (PBS, pH 7.4) and subsequently in PBS. The diameter of the beads can be controlled by altering the stirring speed and can be measured by microscopy.

To introduce the constructed *P. aeruginosa*-agorose beads into the airways of an animal, the animal can be anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). After sterilization, the trachea is cut open, and a volume of 50 µL bead slurry is instilled into the left and right bronchi. The tracheal wound is closed with one to two suture stitches (10-O nylon suture for mice or rats). At a scheduled time (e.g., 3 days after surgery), samples can be collected from the airways for detecting the presence of the bacterium. Mucus and lung tissue samples can be also collected for the

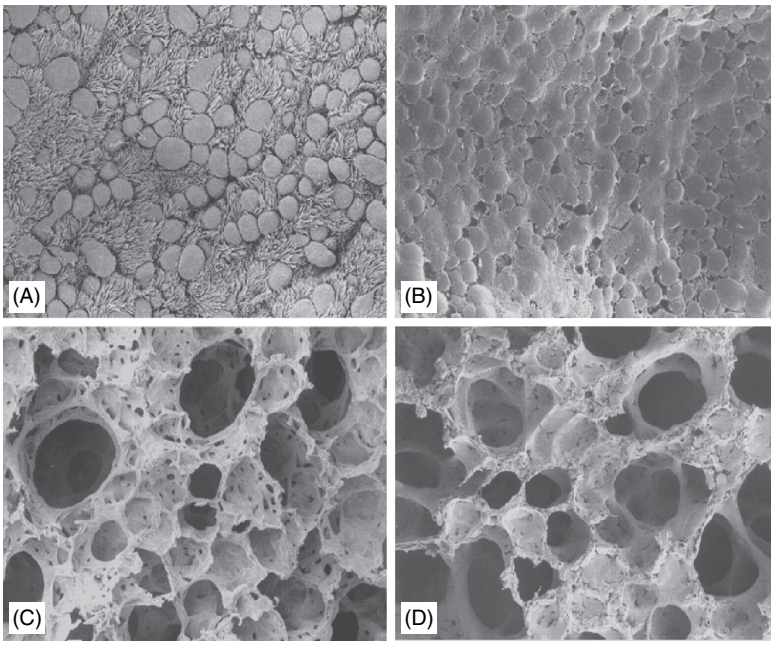


Figure 16.10. Morphological changes in the lung of cystic fibrosis. (A) Scanning electron micrographs of the surface of the respiratory epithelium from a terminal bronchiole in an 11-month-old wildtype mouse. Note the numerous ciliated and nonciliated cells. (B) Terminal bronchiole from a *Cftr*$^{-/-}$ littermate. Respiratory epithelium is encrusted in mucus-like material. (C) Alveoli from the wildtype animal. (D) Alveoli from the affected animal. Distal airways were caked with mucus-like material. Original magnifications: ×1000 (A,B); ×650 (C,D). (Reprinted with permission from Durie PR et al: Characteristic multiorgan pathology of cystic fibrosis in a long-living cystic fibrosis transmembrane regulator knockout murine model, *Am J Pathol* 164:1481–93, copyright 2004.)

detection and analysis of cytokines by biochemistry. Structural changes in the airways can be assessed by microscopy. It is important to note that this model is merely a model of *P. aeruginosa* infection, which often occurs in human patients with cystic fibrosis and exhibits certain features of cystic fibrosis. However, it is not a realistic cystic fibrosis model induced by the mutation of the cystic fibrosis transmembrane regulator gene.

Conventional Treatment of Cystic Fibrosis [16.11]. Cystic fibrosis can be treated with approaches which enhance the function of CFTR and relieve the symptoms of the disorder. Several types of chemical compound, including phenylbutyrate, 8-cyclopentyl-1,3-dipropylxanthine (CPX), and genistein have been tested in previous investigations. These agents may serve as chaperones that promote the deployment of CFTR to the cell membrane. Agents that stimulate chloride secretion (e.g., uridine triphosphate) and inhibit sodium absorption (e.g., amiloride) have also been considered and tested for the treatment of cystic fibrosis. However, few conventional approaches are available for the removal of the causative factors of the disorder.

Cystic fibrosis is associated with life-threatening alterations, such as bronchial constriction and obstruction by inflammatory cells and mucus. A critical issue for treating cystic fibrosis is to relieve bronchial obstruction. Bronchodilators, such as epinephrine, are often

used to achieve such as goal. As discussed on page 750 of this chapter, cystic fibrosis is often associated with bacterial infection. In such a case, antibiotics should be given to control infection. In addition, glucocorticoids are often used to control inflammatory reactions in cystic fibrosis. In the end-stage of cystic fibrosis, it is usually necessary to carry out lung transplantation. However, the long-term survival of lung transplants is often disappointing.

Molecular Engineering [16.12]. Cystic fibrosis can be genetically treated with two approaches: to remove the causative factors and reduce symptoms. Since CFTR gene mutation and deletion are major causes of cystic fibrosis, one strategy for the treatment of cystic fibrosis is to restore the structure and function of the CFTR gene. For the last two decades, numbers of investigations have been carried out to test the effectiveness of CFTR gene transfer into the respiratory system of cystic fibrosis. Limited clinical trials have been carried out. These investigations have shown promising results. However, it is often difficult to achieve long-term therapeutic effects by gene transfer. Furthermore, the effectiveness of the CFTR gene transfer, which requires viral gene-carriers, is limited by local immune reactions to the viral factors.

Another approach is to reduce inflammatory reactions, which often occur in cystic fibrosis. Inflammation is a major factor that contributes to the obstruction of bronchi in cystic fibrosis. As for the treatment of asthma, the overexpression of the glucocorticoid receptor gene by gene transfer is a potential therapeutic approach for reducing inflammatory reactions in cystic fibrosis. Since bronchial constriction and obstruction are major causes of cystic fibrosis-associated symptoms, the transfer of genes encoding bronchodilator proteins is a potential approach for the treatment of cystic fibrosis. A candidate gene is the β_2-adrenergic receptor gene. The upregulation of the β_2-adrenergic receptor induces bronchial dilation and relieves airway obstruction.

Pulmonary Hypertension

Pathogenesis, Pathology, and Clinical Features

Primary Pulmonary Hypertension [16.13]. *Primary pulmonary hypertension*, also known as idiopathic pulmonary hypertension, is a disorder characterized by an increase in pulmonary arterial blood pressure. The term "primary" or "idiopathic" is used because the pathogenic causes of pulmonary hypertension are poorly understood. There are other types of pulmonary hypertension, such as hypoxia- and pulmonary embolism-induced hypertension. Primary pulmonary hypertension is diagnosed only if these types of pulmonary hypertension are excluded.

Although the cause of primary pulmonary hypertension remains unknown, there are several hypothetical mechanisms for the pathogenesis of the disorder. One of the possible causes is disordered regulation of the arterial basal tone or contractility. In pulmonary hypertension, the basal tone of the pulmonary arteries is increased compared to that in the general population. The contribution of altered arterial contractility is supported by the observation that a treatment with vasodilators reduces pulmonary arterial blood pressure. However, the exact cause of disordered arterial contractility remains poorly understood. Another possible cause of primary pulmonary hypertension is the obstruction of pulmonary arteries by unrecognized thromboemboli. Such an obstruction induces elevation in the resistance to the right ventricle, stimulating the right ventricle to increase its contractility. As a result, pulmonary hypertension occurs.

In pulmonary hypertension, pathological examinations exhibit several changes in the structure of pulmonary arteries. These include intimal hyperplasia and wall hypertrophy of pulmonary arteries, a reduction in the density of small pulmonary arteries and capillaries, thickening of endothelial cells and basement membrane of capillaries, and occasionally atherosclerotic plaques in large pulmonary arteries. Pulmonary hypertension is often associated with enlarged right atrium and hypertrophied right ventricle. These changes are attributed to the increase in the workload of the right ventricle resulting from pulmonary hypertension. When the right ventricle is unable to overcome the resistance of the pulmonary arteries and eject sufficient blood into the pulmonary arteries, excessive blood is retained in the right atrium, resulting in the enlargement of the right atrium. The treatment for primary pulmonary hypertension is similar to that for hypoxic pulmonary hypertension (see next section).

Hypoxic Pulmonary Hypertension [16.14]. Hypoxia can induce rapid contraction of smooth muscle cells in the pulmonary arteries of mammals, resulting in acute pulmonary hypertension. Hypoxia is defined as a reduction in the oxygen concentration (<21%). The level of atmospheric oxygen changes in proportion to the altitude. An elevation of 1000 m in altitude is associated with a reduction of about 2% in oxygen concentration. The oxygen level at the altitude 5000 m is about 10%. The exposure of mammals to such an altitude induces acute pulmonary hypertension (Fig. 16.11). The severity of hypertension is proportional to the decrease in the oxygen concentration. Mammals may not survive in an environment with an oxygen level lower than 5%.

It is important to note that, while hypoxia directly induces pulmonary arterial constriction, it exerts an opposite effect on the contractility of the systemic arteries. In the pulmonary arterial system, a reduction in the oxygen level results in a decrease in the air ventilation-to-blood perfusion ratio. As discussed on page 739, the pulmonary system 739 intends to maintain a constant air ventilation-to-blood perfusion ratio and thus to reduce blood perfusion by activating smooth muscle contraction. In the systemic arterial system,

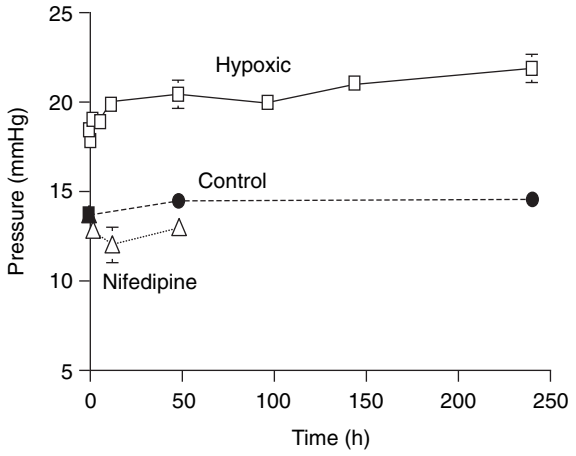


Figure 16.11. Changes in pulmonary arterial blood pressure in hypoxic pulmonary hypertension.

hypoxia is often associated with an increase in metabolic wastes, such as CO_2, H^+, and adenosine. These metabolites induce smooth muscle relaxation in the systemic arteries. However, the effect of these metabolites cannot overcome the constrictive effect of hypoxia in the pulmonary arteries.

Hypoxic pulmonary hypertension can induce rapid pathological changes in the pulmonary arteries. Primary changes include early bleb formation in the endothelial cells (Fig. 16.12), transient edematous swelling and disorganization of the medial elastic laminae (Chapter 16 opening Figure), and progressive hyperplasia of the pulmonary arterial smooth muscle cells and hypertrophy of the pulmonary arterial wall (Fig. 16.13). These are considered adaptive alterations in response to a rapid increase in the pulmonary arterial blood pressure and the stretching tensile stress in the vessel wall. In the presence of hypertension suppressors, such as nifedipine, these pathological changes were significantly inhibited in association with reduced pulmonary arterial blood pressure. These observations support the role of hypertension, but not hypoxia, in the induction of pulmonary arterial pathological changes. When hypoxia is removed, the pathological changes can be gradually recovered. However, the time required for recovery is significantly longer than the time required for the development of the pathological changes.

Rats and mice are often used to induce experimental hypoxic pulmonary hypertension. A hypoxic environment can be created by using a hypoxic chamber with a controlled flow of oxygen and nitrogen. It is necessary to install an oxygen sensor and a CO_2 sensor to monitor the concentrations of oxygen and CO_2, respectively. Animals can be placed in the chamber for a desired period. Pulmonary arterial blood pressure usually starts to increase within 5 min, continues to increase for about 5 days, and reaches a plateau after 5 days. The pulmonary arterial blood pressure can be monitored in living animals by implanting a catheter into the pulmonary arterial trunk or the right ventricle via the jugular vein and the right atrium.

Conventional Treatment of Pulmonary Hypertension [16.13, 16.14]. The principle of treating pulmonary hypertension is to induce arterial dilation and reduce the pulmonary arterial blood pressure. Vascular smooth muscle relaxants are often used to achieve such a goal. Common smooth muscle relaxants include direct smooth muscle relaxants (e.g., nitroprusside and nitroglycerine), β-adrenergic agonists (e.g., isoproterenol), α-adrenergic blockers (e.g., phentolamine and phenoxybenzamine), and calcium channel blockers (e.g., nifedipine). These agents are effective for the temporary relief of pulmonary hypertension. When pulmonary arterial embolism is the cause of pulmonary arterial hypertension, patients should be treated with anticoagulants.

Molecular Therapies for Pulmonary Hypertension. Primary pulmonary hypertension is a result of pulmonary arterial constriction due to hyperactivity of smooth muscle cells. Thus, the principle of treating pulmonary hypertension by molecular engineering is to relax smooth muscle cells, dilate pulmonary arteries, and reduce pulmonary arterial blood pressure. Because the genes responsible for the hyperactivity of smooth muscle cells remain poorly understood, there are few molecular engineering approaches available for removing the causative factors. Several genes encoding vasodilator proteins have been used in experimental models for the treatment of pulmonary hypertension. These include the nitric oxide synthase gene, the prostaglandin synthase gene, and the prepro-calcitonin gene-related peptide gene. These genes are briefly described as follows.

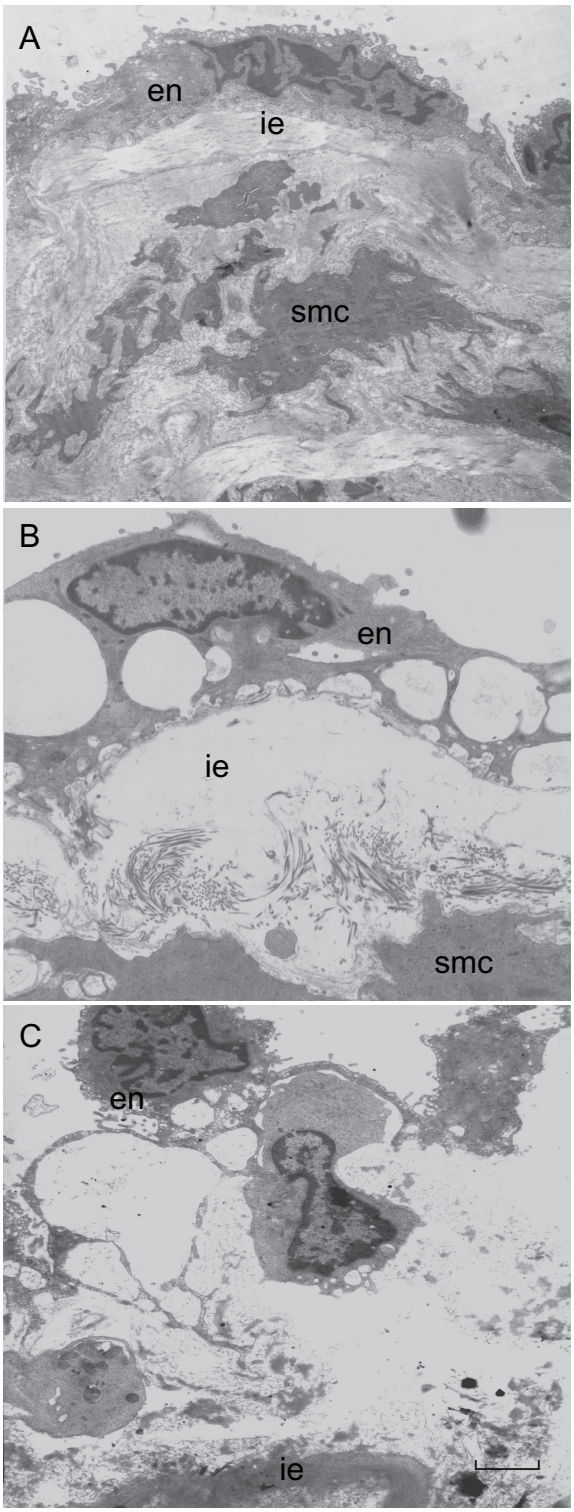


Figure 16.12. Electron micrographs of pulmonary arteries in hypoxia-induced pulmonary hypertension. (A) Control. (B) 12 hours of hypoxia. (C) 30 days of hypoxia. en: endothelial cell. ie: internal elastic lamina. smc: smooth muscle cell. Scale: 1 μm.

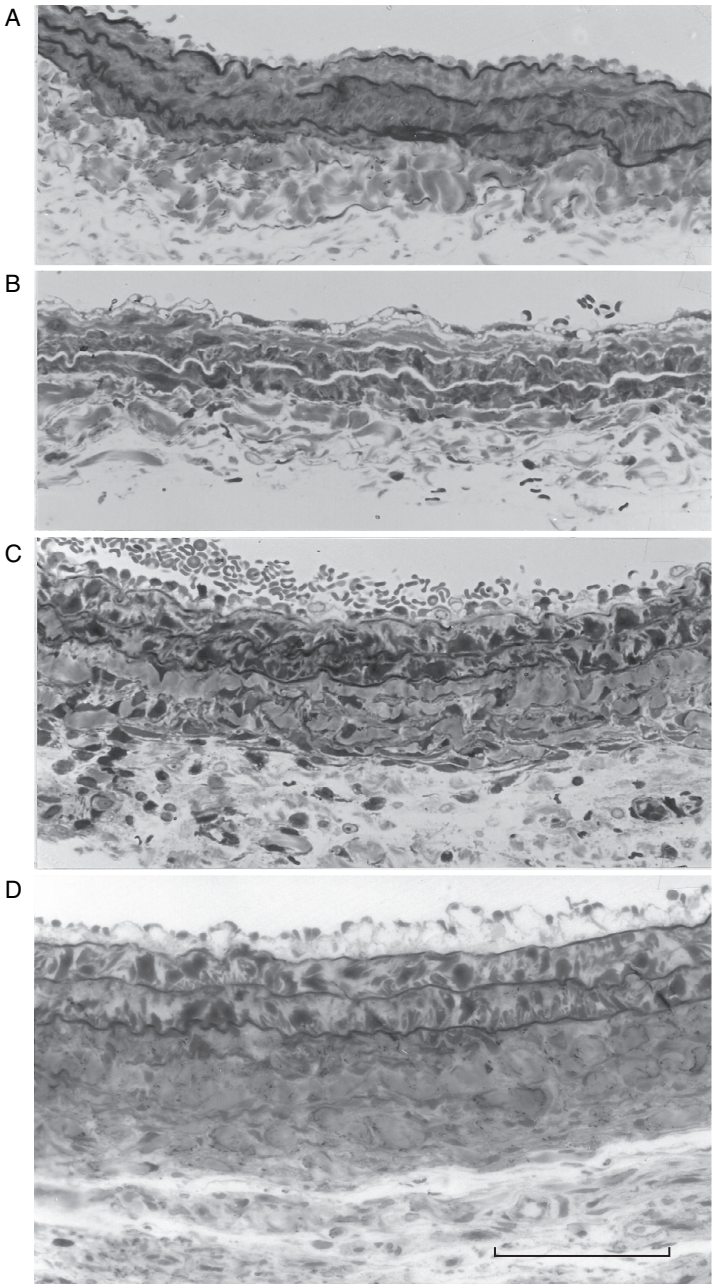


Figure 16.13. Pulmonary arterial structure in hypoxia-induced pulmonary hypertension. (A) Control. (B) 12 hours of hypoxia. (C) 10 days of hypoxia. (D) 30 days of hypoxia. Scale: 100 µm.

Nitric Oxide Synthase Gene [16.15]. As discussed in Chapter 15, nitric oxide synthase catalyzes the formation of nitric oxide from L-arginine. Nitric oxide induces rapid relaxation of smooth muscle cells, dilation of arteries, and reduction in arterial blood pressure. The overexpression of the nitric oxide synthase gene enhances the translation of nitric

oxide synthase and therefore the production of nitric oxide. Experimental investigations have shown that adenovirus-mediated transfer of the nitric oxide synthase gene into the lung of the rat with pulmonary hypertension results in a decrease in pulmonary arterial blood pressure and a reduction in the responsiveness arterial smooth muscle cells to vasoconstrictors, such as angiotensin II and endothelin-1. In contrast, the deletion of the nitric oxide synthase gene in mice is associated with profound pulmonary hypertension. The transfer of the nitric oxide synthase gene into the lung of nitric oxide synthase-deficient mice results in a reduction in the pulmonary arterial blood pressure. These observations have demonstrated that the nitric oxide synthase gene is a potential therapeutic gene for the treatment of pulmonary hypertension.

Prostaglandin I2 Synthase Gene [16.16]. Prostaglandins are fatty acid-derived molecules that act on cell membrane receptors and induce relaxation of vascular smooth muscle cells and dilation of arteries. Prostaglandin I2 synthase, also known as prostacyclin synthase, PGI2 synthase, and PGIS, is an enzyme of 500 amino acids and about 57 kDa. This enzyme is a member of the cytochrome P450 superfamily of enzymes and is expressed in the blood vessels (endothelial cells, smooth muscle cells), lung, ovary, brain, intestine, and testis. Prostaglandin I2 synthase catalyzes the conversion of prostglandin H2 to prostacyclin (prostaglandin I2), which is a potent vasodilator and inhibitor of platelet aggregation and atherogenesis. The overexpression of the prostaglandin I2 synthase gene enhances the formation of the prostaglandin I2 synthase protein and prostaglandins as well. Experimental studies have demonstrated that mice with genetically induced overexpression of the prostaglandin I2 synthase gene exhibit increased production of pulmonary prostaglandins. When exposed to hypoxia, which induces pulmonary hypertension, the transgenic mice are more resistant to hypoxic hypertension than control mice without overexpressing the prostaglandin I2 synthase gene. These observations suggest that the prostaglandin I2 synthase gene can be considered a potential gene for the treatment of human pulmonary hypertension.

Preprocalcitonin-Related Peptide Gene [16.17]. The preprocalcitonin gene-related peptide is a precursor for calcitonin-related peptide (CGRP) and is expressed in the nerve and endocrine cells of the airways. CGRP, also known as *calcitonin gene-related peptide 2*, CGRP2, CGRP II, β-type CGRP, is a protein of 127 amino acids and about 14-kDa. This protein can act on its receptors in the vascular smooth muscle cell and induce dilation of the pulmonary arteries. In pulmonary hypertension induced by hypoxia, the level of CGRP is reduced, an alteration potentially contributing to the development of pulmonary hypertension. Thus, the enhancement of CGRP production and activation is a potential approach for the treatment of pulmonary hypertension. Experimental investigations have demonstrated that, in the animal model of hypoxia-induced pulmonary hypertension, the overexpression of the prepro-CGRP gene results in an increase in the production of CGRP and a reduction in the pulmonary arterial blood pressure in association with an attenuation of pulmonary arterial and right ventricular hypertrophy. These preliminary investigations suggest that the prepro-CGRP gene may be considered a potential gene for the treatment of pulmonary hypertension. Although hypoxia-induced hypertension does not assemble human primary pulmonary hypertension, vasodilation is a general approach for relieving the symptoms of pulmonary hypertension regardless the causative factors.

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