Ultrastructural Studies of the Airway Epithelium in Airway Diseases

EYMAN SHEBANI
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Abstract

Ultrastructural studies of airway epithelium in airway disease are important for diagnosis and understanding the underlying pathology which helps clinicians to improve the patients' treatment.

Airway biopsies from a 5-month old boy with respiratory problems and gastro-oesophageal reflux were studied by transmission electron microscopy (TEM). The tracheal columnar cells showed accumulation of lamellar bodies, indicative of lysosomal storage disease. The patient was diagnosed with Gaucher disease type 2.

Shedding of airway epithelial cells is commonly found in asthma. The attachment of these cells to the basal lamina was investigated by TEM of biopsies from patients with asthma and healthy controls. The contact area between columnar cells and basal lamina in asthmatics was significantly less than in controls. Attachment of columnar cells to the basal lamina occurs mainly indirectly, via desmosomal attachment to basal cells.

Primary ciliary dyskinesia (PCD) is a congenital disease. It is important to differentiate PCD from acquired (secondary) ciliary dyskinesia (SCD). The number of dynein arms determined by TEM was 1.5 and 1.4 for outer and inner dynein arms, respectively in PCD, versus 7.9 and 5.2 for controls and 8.1 and 5.9 in SCD. Compared to PCD patients, SCD patients have more structurally abnormal cilia. A significant difference was found in orientation of the central microtubule pair between PCD and SCD, but also overlap.

Leukotriene receptor antagonists are a new treatment for asthma. Both corticosteroids and montelukast caused apoptosis and necrosis of airway epithelial cells, and reduced the expression of intercellular adhesion molecule-1. Treatment of cells with tumor necrosis factor-α or interferon-γ reduced the fraction of the lateral cell membrane occupied by desmosomes and this effect was counteracted by corticosteroids.

Keywords: asthma, cilia, corticosteroids, cytokines, desmosomes, Gaucher’s disease, montelukast, primary ciliary dyskinesia, transmission electron microscopy

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urn:nbn:se:uu:diva-6632 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-6632)
To

My parents,

Mahmoud

and my children

He who hears, forgets; he who sees, remembers; he who does, knows.

Ancient Chinese Philosopher
List of papers

This thesis is based on the following papers referred to in the text by their Roman numerals:


IV Andersson K, Shebani E, Makeeva N, Servetnyk Z, Borg LAH, Roomans GM. Effects of corticosteroids and leukotrien receptor antagonists on airway epithelial cells (submitted for publication).
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<tr>
<td>ASL</td>
<td>Airway surface liquid</td>
</tr>
<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>CBF</td>
<td>Ciliary beat frequency</td>
</tr>
<tr>
<td>COR</td>
<td>Ciliary orientation</td>
</tr>
<tr>
<td>Cys-LT</td>
<td>Cysteinyl leukotriens</td>
</tr>
<tr>
<td>Der p 1</td>
<td>Dust mite <em>Dermatophagoides pteronyssinus</em> allergen</td>
</tr>
<tr>
<td>ECGS</td>
<td>Endothelial cells growth medium with calf serum</td>
</tr>
<tr>
<td>ECP</td>
<td>Eosinophilic cationic protein</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>EMEM</td>
<td>Eagle’s minimal essential medium</td>
</tr>
<tr>
<td>FACS</td>
<td>Fluorescence-activated cell sorter</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced expiratory volume in 1 second</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte-macrophage colony stimulating factor</td>
</tr>
<tr>
<td>16HBE</td>
<td>Human bronchial cell line</td>
</tr>
<tr>
<td>HUVEC</td>
<td>Human umbilical vein cells</td>
</tr>
<tr>
<td>ICAMs</td>
<td>Intercellular adhesion molecules</td>
</tr>
<tr>
<td>INF-γ</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LT</td>
<td>Leukotriene</td>
</tr>
<tr>
<td>LTRA</td>
<td>Leukotriene receptor antagonist</td>
</tr>
<tr>
<td>MAPs</td>
<td>Microtubule associated proteins</td>
</tr>
<tr>
<td>NHS</td>
<td>Normal human serum</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PAS</td>
<td>Periodic acid-Schiff</td>
</tr>
<tr>
<td>PC₂₀</td>
<td>Provocation concentration that reduces FEV₁ by 20%</td>
</tr>
<tr>
<td>PCD</td>
<td>Primary ciliary dyskinesia</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak expiratory flow</td>
</tr>
<tr>
<td>vPEF%</td>
<td>Percentage of peak expiratory flow variability</td>
</tr>
<tr>
<td>PFT</td>
<td>Pulmonary function tests</td>
</tr>
<tr>
<td>SCD</td>
<td>Secondary ciliary dyskinesia</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris-buffered saline</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>Z-VAD-fmk</td>
<td>Caspase inhibitor</td>
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INTRODUCTION

The airway epithelium

The major function of the respiratory epithelium was once thought to be only that of a physical barrier. However, it is much more and constitutes the interface between the internal milieu and the external environment. It also is a primary target for toxic, chemical and microbial agents. Thus, damage to the epithelium may contribute substantially to inflammation, and the bronchoconstriction and oedema seen in asthma and a number of other respiratory diseases. In addition to its barrier function, the epithelium also has a secretory function, since it can produce an incredibly diverse array of lipid mediators, growth factors, bronchoconstricting peptides, chemokines, and cytokines (Knight & Holgate, 2003).

The conducting part of the airways is lined by epithelium and the airway wall contains epithelium, connective tissue, smooth muscle, cartilage and mucus-producing glands (McKay & Hogg, 2002). In the normal human airways the surface epithelium is on average 50 µm thick, and rests on a roughly contoured basement membrane (Jeffery & Brain, 1988). The airway epithelium is a ciliated pseudostratified columnar epithelium containing three main cell types, the ciliated cells, non-ciliated cells (both these cell types are tall columnar cells), and basal cells. However, more distally in the respiratory tract the epithelium changes towards a simple cuboidal epithelium (Engelhardt et al., 1994).

Ciliated cells are the predominant cell type within the airways, accounting for over 50% of all epithelial cells (Spina, 1998). Ciliated cells are terminally differentiated columnar cells, which are thought to originate from basal or secretory cells. Typically, ciliated epithelial cells possess up to 300 cilia/cell and numerous mitochondria immediately beneath the apical surface, highlighting their main function, namely to remove particulate matter by means of the mucociliary stairway (McDowell et al., 1978; Harkema, 1991; Knight & Holgate, 2003). They also transport water and ions, and release macromolecules. Ciliated cells are found down to the terminal bronchiole. Usually they reach the basement membrane, extending slender proc-
esses that reach the basal lamina (Rennard et al., 1994) but they do not form hemidesmosomes to attach to the basal lamina (Hasleton, 1996).

The non-ciliated cells (known also as the secretory cells) comprise goblet, serous, Clara, neuroendocrine and presecretory cell types. Secretory cells, which comprise 15-25% of the bronchial and nasal epithelium, are primarily involved in the production of airway secretion (the mucus) in which various particles, including viruses and bacteria, can be trapped (Mills et al., 1999; Harkema, 1991). Clara cells are thought to produce bronchiolar surfactant apoproteins A and B. In addition Clara cells are believed to metabolise xenobiotic compounds, and recently it has been suggested that they serve as a progenitor for both ciliated and mucus secreting cells (De Water et al., 1986; Hong et al., 2001). Two layers of fluid cover the epithelium: the airway surface liquid (ASL) that covers the cells and in which the cilia bathe and a superficial mucus layer on the tip of the cilia (Liedtke, 1989; Welsh, 1987).

Below the layer of columnar cells a layer of basal cells is present that plays a role in the attachment of the columnar cells to the airway basement membrane (Davies & Devalia, 1992). Flint and colleagues suggested that epithelial basal cells may have a function other than progenitor cells for epithelial renewal (Flint et al., 1986). The question as to whether basal cells are the progenitors of columnar cells (Welsh, 1987; Boers et al., 1998) or not (Davies & Devalia, 1992) is still under debate. There is a direct correlation between the thickness of the epithelium and the number of basal cells as well as the percentage of columnar cell attachment to the basement membrane via the basal cell (Hicks et al., 1997). Within the epithelium, basal cells are the only cells that are firmly attached to the basement membrane via hemidesmosomal complexes (Evans & Plopper, 1988). Studies in animals have shown that basal cells form hemidesmosome attachments with the basal lamina and desmosome attachments with adjacent cells (Laitinen & Laitinen, 1994a). In a study by Erjefält and coworkers it was suggested that the basal cells may be well equipped to take over a barrier structure promptly when the columnar epithelial cells are shed. Such a property of airway basal cells would be important in health and disease (Erjefält et al., 1997). In addition to their progenitor and structural roles, basal cells are also thought to secrete a number of bioactive molecules including neutral endopeptidase, 15-lipoxygenase products and cytokines (Knight & Holgate, 2003).

Bronchial epithelial cells express several molecules on their surface, which are involved in the adhesion and activation of leukocytes. Airway epithelial cells produce a number of mediators, such as nitric oxide (NO), that suppress airway contractility (Campbell et al., 1993; Felley-Bosco et al., 1994). Epithelial cells are also capable of producing proinflammatory cyto-
kines such as GM-CSF, IL-6, and IL-8 and eotaxin by which eosinophils, neutrophils and lymphocytes can be attracted and activated (Erger & Casale, 1995; Lilly et al., 1997; Marini et al., 1992).

Basement membrane

All epithelial cells in contact with subjacent connective tissue have, at their basal surfaces, a sheetlike extracellular structure called the basal lamina. This structure is visible only with the electron microscope, where it appears as a dense layer, 20-100 nm thick, consisting of a delicate network of fine fibrils (lamina densa). The term basement membrane is used to specify a periodic acid-Schiff (PAS) positive layer, visible with the light microscope beneath the epithelium. The basement membrane is usually formed by the fusion of either 2 basal laminae or a basal lamina and a reticular lamina (Junqueira & Carneiro, 2003). The epithelial cells of the airway epithelium secrete the upper layer of the basement membrane, the lamina densa, which consists predominantly of type IV collagen and type V laminin. The thicker lower layer, the lamina reticularis, is synthesized primarily by subepithelial fibroblasts and consists of types III and V collagen and fibronectin (Paulsson, 1992). The basement membrane acts as a barrier between the surface epithelium and the underlying connective tissue. In addition it acts as an anchor for the epithelium and facilitates adhesion and migration of epithelial cells (Terranova et al., 1980). Recent studies have demonstrated the presence of pores within the basement membrane, which may allow passage of infiltrating cells (Howat et al., 2001).

Cilia

Cilia are eukaryotic cell organelles that play important roles including cell motility, transport of mucus, other fluids, and even other cells, and function in communication with the extracellular environment (Pan et al., 2005). There are two types of cilia: motile cilia, which propel a single cell through liquid or move fluid across the surface of a layer of cells, and immotile cilia, which usually serve as sensors (Chodhari et al., 2004). In humans, motile cilia are found lining the upper and lower respiratory tract, sinuses, middle ear, the ependyma of the brain, the ductuli efferentes of males, and the female oviduct, and they are found in the uterus. Furthermore, cilia are morphologically similar to flagella of spermatozoa (Meeks & Bush, 2000). Motile cilia are also present in Hensen’s node, which are responsible for the unidirectional flow of fluid on the back of the embryo, which determines sidedness (Nonaka et al., 2002). Ciliated cells are a part of the mucosal pseudostratified columnar epithelium lining the respiratory tract. Each cili-
ated cell carries approximately 200 cilia on its surface. The function of these cilia is to beat in a coordinated manner to provide normal clearance of mucus and other debris from the airways. The normal ciliary beat frequency is 11-16 Hz (Bush et al., 1998; Cowan et al., 2001).

Ultrastructure of cilia

Cilia are highly complex organelles. More than 250 proteins are involved in the formation of cilia. The majority of these are components of a specific axoneme structure. There are many other proteins that are important for ciliary assembly, initiation, orientation, and control of ciliary activity (Dutcher, 1995). The core of the motile cilium is the axoneme, which consists of nine peripheral microtubule doublets surrounding a central pair of singlet microtubules (9+2). Each of the microtubular doublets is constructed from heterodimers of α- and β-tubulin, assembled into the 13 protofilaments of the A tubule and the 11 protofilaments of the B tubule. The microtubules of the central pair are composed of 13 protofilaments of tubulin and have approximately the same orientation as the central pair of adjacent cilia, which is important in the production of a coordinated ciliary waveform (Meeks & Bush, 2000). Each cilium is anchored to a basal body in the cytoplasm near the plasma membrane. This structure is composed of nine microtubule triplets. Each microtubule doublet is connected to the adjacent doublets by nexin links and to the central pair by radial spokes (Cowan et al., 2001).

Figure 1. Diagram of ultrastructural components of a cilium, as seen in cross section (Meeks & Bush, 2000).
There are also several microtubule associated proteins (MAPs) which play structural and functional roles. Among the MAPs most studied is dynein, which forms outer and inner dynein arms, structures which are absent or abnormally small in most PCD patients. Dynein is a high molecular weight protein that belongs to the group of mechanochemical ATPases. Outer dynein arms are thought to regulate beat frequency and inner arms to regulate beat waveform (Meeks & Bush, 2000).

Ciliary activity has been shown to occur via ATP hydrolysis by dynein heavy chains, which causes a sliding of the A microtubule relative to the B microtubule. This results in bending of the cilia (Cowan et al., 2001).

Other MAPs within the the ciliary axoneme include proteins associated with the radial spoke complexes, the central pair apparatus, and the nexin links (Yang et al., 2001).

Lysosomes

Like any other cell in the human body, the airway epithelial cell is composed of two basic parts: cytoplasm and nucleus. The cytoplasm is separated from the extracellular environment by the plasma membrane and composed itself of a matrix (cytosol), in which are embedded the organelles, the cytoskeleton, and deposits of carbohydrates, lipids, and pigments. Lysosomes are discrete particulate organelles that are specialized secretory products of the endoplasmic reticulum and Golgi apparatus. Lysosomes are membrane-limited vesicles that contain a large variety of hydrolytic enzymes that destroy engulfed materials. Although the nature and activity of lysosomal enzymes vary depending on the cell type, the most common enzymes are acid phosphatase, ribonuclease, deoxyribonuclease, proteases, sulfatases, lipases, and β-glucuronidase. Lysosomes, which are spherical, range in diameter from 0.05 to 0.5μm and present a uniformly granular, electron dense appearance in electron micrographs. Lysosomes are abundant in cells exhibiting phagocytic activity (Johnson, 1991; Junqueira & Carneiro, 2003). The endosomal-lysosomal system is critical for a diverse set of cellular functions, including processing of intracellular proteins, down-regulation of plasma membrane receptors, and protein turnover. Lysosomes, an essential component of this system, are important for degradation of various cellular constituents (Szweda et al., 2003), and consequently many diseases have been ascribed to deficiencies of lysosomal enzymes. In most of these diseases, a specific lysosomal enzyme is absent or inactive, and certain molecules are not digested. As a result, these substances accumulate in the cells, interfering with their normal functions (Junqueira & Carneiro, 2003). Storage material such as lamellar inclusions with specific patterns, clefts, spicules, vacuoles with particular elements, is a useful morphological diagnostic marker in the lysosomal and peroxisomal disorders (Ceuterick de Groote & Martin, 1998).
The most prevalent lysosomal storage disease, Gaucher disease, is caused by the inefficient folding and trafficking of certain variants of β-glucocerebrosidase (Cohen & Kelly, 2003).

Adherent structures

The plasma membrane of the epithelial cell forms specialized adhesion complexes for cell-cell contacts that are important for the organisation and behavior of the cell. These contacts make it possible for the epithelium to fulfill its barrier function, provide anchoring sites both for intermediate filaments and for actin filaments and are also thought to be important in the generation of different intracellular signals (Troyanovsky, 1999). There are two types of cell contacts: cell-cell contacts, and cell-matrix contacts. Cell-cell contacts include (from apex to the base of the cell) tight junctions, intermediate junctions, desmosomes, and gap junctions. Cell-matrix contacts include hemidesmosomes and focal adhesions.

Cell to cell contacts

Tight junctions

Epithelial cells are connected by tight junctions but the structure and function of these junctions varies between different types of cells and between different parts of the airway. The transepithelial resistance decreases in the distal airways (Welsh, 1987). Tight junctions (zonulae occludentes) are located at the apicolateral borders of the epithelial cells and regulate the passage of water, ions, neutral molecules and inflammatory cells through the paracellular pathway (fluid filled spaces between the cells) (Godfrey, 1997). The outer leaflet membrane of two adjacent cells creates a series of apparent fusions with completely obliterated intercellular spaces (Anderson, 2001). In 1963, Farquhar and Palade were the first to describe the tight junction and its functions using electron-dense markers (Farquhar & Palade, 1963). When tight junctions are visualized by freeze-fracture electron microscopy, they seem to be composed of a branching network of sealing strands that completely encircles the apical end of each cell in the epithelial sheet (Alberts et al., 2002). The major proteins thought to play a significant role in the function of tight junctions are the transmembrane proteins, claudin, occludin, junctional adhesion molecule, and a number of associated intracellular proteins such as ZO-1, ZO-2, and ZO-3 (Tsukita et al., 1999). These proteins connect to actin filaments that make up the cytoskeleton (Saitou et al., 1998). Claudins have been under intensive investigation recently as they seem to
form the backbone of the tight junctions. They are also responsible for the obliteration of the intercellular space between two cells (Tsukita & Furuse, 1999). Tight junctions play a major role in maintaining the integrity of the epithelium (Elia et al., 1988). Wan and coworkers have reported that the cysteine proteinase allergen Der p 1 from fecal pellets of the house dust mite Dermatophagoides pteronyssinus causes disruption of intercellular tight junctions, which are the principal components of the epithelial paracellular permeability barrier (Wan et al., 1999). Tight junctions of airway epithelia exposed to chronic inflammation may exhibit parallel changes in the barrier function to both solutes and ions. These changes in permeability are cytokine-induced (Coyne et al., 2002).

Intermediate junctions

Intermediate junctions form a continuous belt (zonulae adherentes) just below the tight junctions, encircling each of the interacting cells in the sheet. Their most obvious feature is a contractile bundle of actin filaments running along the cytoplasmic surface of the junctional plasma membrane. These actin filaments are joined from cell to cell by transmembrane adhesion proteins called cadherins. The intracellular tails of the cadherins bind to anchor proteins (such as \( \alpha \) catenin, \( \beta \) catenin and \( \alpha \) actinin) that tie them to actin filaments (Alberts et al., 2002).

Desmosomes

The existence of a structure specialized for cell adhesion was first observed by Bizzozero in 1870 as densities at the end of long processes joining two cells. It was not until 1954 that these densities were first observed with an electron microscope by Porter, and were shown to be what are now known as spot desmosomes (maculae adherentes) (Arn & Staehelin, 1981). They sit along the lateral border of columnar cells and between basal and columnar cell layers. They are considered to be the major structural adhesives at the plane of cleavage between columnar and basal cells (Evans et al., 1989). Desmosomes are involved in maintaining the structural and functional interaction of adjoining cells (Legan et al., 1992). Desmosomes are transmembrane molecules belonging to the cadherin family of calcium-dependent adhesion molecules (desmogleins and desmocollins) that are linked to the intermediate filament cytoskeleton, presumably through plakoglobin and desmoplakin (Burdett, 1998). Desmosomes are made up of several proteins. The glycoproteins, desmocollin and desmoglein, are involved in desmosomal adhesion while the armadillo proteins, plakoglobin and plakophilin, are needed for the adhesive function of glycoproteins and for linking glycoproteins to desmoplakin. Desmoplakin provides linkage between the desmosomal plaque and the keratin intermediate filaments that extend into the cyto-
Desmosomes are now being regarded, not as static and inert structures, but as membrane specializations linked to systems involved in cell-cell communication as well as adhesion (Burdett, 1998). Besides the fact that desmosomes provide the tissues with mechanical strength, they have also recently been recognized as sensors that respond to environmental and cellular cues by modulating their assembly state and, possibly, their signalling functions (Green & Gaudry, 2000).

Desmosomes have, so far, been implicated in three main disease types: autoimmune diseases that involve desmosome components (such as Pemphigus vulgaris and Pemphigus foliaceus), congenital diseases that affect intracellular calcium channels (such as Hailey-Hailey disease and Darier disease), and congenital diseases that directly affect desmosomal structural components (McMillan & Shimizu, 2001). Autoantibodies to desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3), causing breakdown of the desmosomes, seem to be responsible for the clinical manifestations in a variety of skin diseases such as Pemphigus vulgaris, in which blisters arise in layers corresponding to the position of Dsg1 or Dsg3 in the epidermis or oral cavity (Ishii & Green, 2001). Experimental studies in our laboratory have pointed to a possible role of desmosomes in epithelial shedding in the airways. Poly-L-arginine, a functional analogue of the eosinophil granule derived major basic protein (MBP), which is elevated in the serum of asthma patients, caused a reduction in desmosomes in cultured human bronchial epithelial cells (Shahana et al., 2002). Also in a recent ultrastructural study it was found that in the airway epithelium of asthma patients, desmosomal contacts between epithelial cells were reduced and half-desmosomes were found in the columnar cells (Shahana et al., 2005). Half-desmosomes have been observed in epithelial cells cultured in low calcium medium, where desmosomal proteins were synthesized but not assembled, or formed partially assembled unstable half-desmosomes (Burdett, 1998).
Gap junctions

A considerable body of data suggests that gap junctions (nexus), represent channels that facilitate intercellular communication, thereby modulating growth and development (Carson et al., 1989). Gap junctions are tightly packed aggregations of over 105 intercellular channels that directly connect the cytoplasm of adjacent cells. Intercellular channels allow the transfer of ions, metabolites and messenger molecules less than 1 kDa in size between connected cells, providing mechanisms for coordinating the activities of groups of cells (Simon & Goodenough, 1998). The connexon, the unit gap junction channel, is a pair of hemichannels apposed in the narrow intercellular gap between neighbouring cell membranes. It has been shown that hemichannels are composed of hexamers of connexin proteins (Shibata et al., 2001); in some instances, different connexin proteins can together form one connexon (Kilarski et al., 2001). Gap junctions are not specific for epithelial cells, but are found in most cell types.

Cell to matrix contacts

Hemidesmosomes

Hemidesmosomes are specialized junctional complexes that contribute to the attachment of epithelial cells to the underlying basement membrane. These multiprotein complexes determine cell-stromal coherence and provide cells with cues critical for their polarization, their spatial organization, and for tissue architecture. Hemidesmosomes may not only represent structural adhesion complexes, but they may also serve via the α6β4 integrins as “signaling devices” affecting the cell phenotype (Borradori and Sonnenberg, 1999). Ultrastructurally, hemidesmosomes appear as tripartite structures consisting of an inner and outer plaque and a sub-basal dense plate. The inner hemidesmosomal plaque is composed of the HD1/plectin and BP230 proteins, which are involved in connecting the hemidesmosome to the keratin intermediate filament system. The outer plaque contains the hemidesmosome transmembrane protein α6β4 which mediates binding of the basal cell to the underlying basement membrane by binding to laminin (Nievers et al., 1999). Hemidesmosomes were considered earlier as halves of desmosomes (Garrod, 1993), but although hemidesmosomes and desmosomes have similarities regarding their connection with the cytoskeleton system, they consist of different structural proteins. Hemidesmosomes contain a set of proteins from the integrin family, whereas desmosomes contain proteins from the cadherin family. Hemidesmosomes play an important role in maintaining tissue integrity (Nievers et al., 1999). The importance of these complexes is attested by
the fact that an altered expression of hemidesmosomal constituents results in the pemphigoid blistering disorders of the skin (Borradori and Sonnenberg, 1999).

Focal adhesions

Focal adhesions became an important issue in 1971, when Abercrombie and coworkers discovered that chicken fibroblasts in tissue culture formed dense plaque-like areas in the cellular extensions when migrating. They reported that these were adhesion sites used to move the cell body to new areas (Abercrombie et al., 1971). Focal adhesions are anchoring junctions that also bind cells to the extracellular matrix. The transmembrane adhesion proteins, similar to hemidesmosomes, are integrins. Focal adhesions enable cells to get a hold on the extracellular matrix through integrins that link intracellularly to actin filaments. The extracellular domains of transmembrane integrin proteins bind to a protein component of the extracellular matrix, while their intracellular domains bind indirectly to bundles of actin filaments via the intracellular anchor proteins talin, α actinin, filamin, and vinculin (Alberts et al., 2002).

Non junctional adhesions

All the above mentioned contacts are junctional. The non junctional adhesions (adhesion molecules), are also known as cell surface markers. The adhesion molecules are membrane glycoproteins that mediate adhesion between two cells or between the cell and extracellular matrix (Alvaro, 2000). They are divided into several superfamilies depending on their interaction, e.g. selectins, integrins, cadherins, integral membrane proteoglycans, and Ig-like CAMs (cell adhesion molecules). ICAMs and vascular cell adhesion molecules (VCAMs) induce adhesion of inflammatory cells to the vascular wall (Blankenberg et al., 2003).

ICAM

CD54, more commonly referred to as intercellular adhesion molecule-1 (ICAM-1), can be found on leukocytes and non-haematogenous cells (Sligh et al., 1993). Studies of nasal turbinates showed that ICAM-1 was broadly distributed within the epithelium, on ciliated and basal epithelial cells and in the submucosa on endothelial and inflammatory cells (Shirasaki et al., 2004). It has been demonstrated that epithelial upregulation of ICAM-1, which is involved in interaction with other cells, is present in asthma. The expression of ICAM-1 on human airway epithelial cells is stimulated by inflammatory cytokines, such as IL-1 or tumor necrosis factor alpha (TNF-α) (Tosi et al.,
Active bronchial asthma is associated with the presence of increased levels of soluble ICAM-1 in serum, and corticosteroid therapy caused reduction of these levels (Shiota et al., 1996). Blocking or down-regulating ICAM-1 can interfere with the activation and trafficking of immune and inflammatory cells to the site of inflammation (Sligh et al., 1993). ICAM-1 is greatly over-expressed in inflamed tissues and thereby implicated in the pathogenesis of disease states, such as asthma (Ohkawara et al., 1995).

Figure 2. Summary of cell contacts (Alberts et al., 2002)
Diseases involving the airways

Gaucher disease

Gaucher disease is due to an autosomally recessively inherited deficiency of the lysosomal enzyme glucocerebrosidase, which catalyses the hydrolytic cleavage of glucose from glucocerebroside (Niederau & Häussinger, 2000). This deficiency causes an accumulation of the sphingolipid glucosylceramide in the cells of the reticulo-endothelial system, “Gaucher cells”. This abnormal deposition occurs primarily in the bone marrow, spleen, liver and other organs (Kerem et al., 1996). Gaucher cells resemble macrophages with eccentric, small, oval nuclei, but can be distinguished from these by their abundant cytoplasm with the characteristic “rumpled tissue paper” appearance. These cells stain strongly positive with PAS stain. Electron microscopic views of these cells reveal numerous intracytoplasmic elongated, membrane-bound lysosomes containing the characteristic twisted tubular structures (Carson et al., 1994). Bone marrow biopsy is most useful in the diagnosis of Gaucher disease. Gaucher cells show characteristic bundles of twisted tubular inclusions in the different forms of Gaucher disease (Ceuterick-de Groote & Martin, 1998). Glucocerebrosidase activity can be measured in leukocytes, cultured fibroblasts, lymphoblasts, amniocytes, and chorionic villi samples. However, neither the amount of residual enzyme activity nor the amount of stored lipid reliably differentiates the different types of Gaucher disease (Tayebi et al., 1999).

The disease has been classified into three clinical types based on the presence or absence of central nervous system involvement and disease severity. Type 1 Gaucher disease is the mildest form and is without neurologic involvement (Liu et al., 1998). Type 1 or non-neuropathic Gaucher disease is by far the most common type and often presents with cytopenia, hepatosplenomegaly, or skeletal involvement. The age at onset of symptoms is variable and many individuals with type 1 Gaucher are asymptomatic (Tayebi et al., 1999). Type 1 is especially common among Ashkanazi Jews (Amir & Ron, 1999). Type 2, the most severe form of the disease, is characterised by an early onset of neurologic disease and acute course. The type 3 disease is of intermediate severity with a later onset of neurologic symptoms and a more chronic course (Liu et al., 1998). Often the sole neurologic symptom in type 3 is the slowing and looping of the horizontal saccadic eye movements. Gaucher disease type 3 is overrepresented in parts of Northern Sweden. In recent years, several atypical presentations of Gaucher disease have been recognised, including hydrops fetalis, congenital ichthyosis, car-
diovascular fibrosis and/or calcifications, pulmonary hypertension, hydrocephalus, and parkinsonian symptoms (Tayebi et al., 1999).

The gene for human glucocerebrosidase is located on chromosome 1q21. Over 100 mutations in the glucocerebrosidase gene have been described in the DNA of patients with Gaucher disease (Stone et al., 2000). The substitution of serine for asparagine at amino acid residue 370 (the N370S mutation) is associated with type 1 disease. On the other hand, a substitution of proline for leucine at amino acid residue 444, especially in the homozygous state is associated with type 2 and 3 diseases (Santamaria et al., 1998). The L444P mutation is present in the sequence of the glucocerebrosidase pseudogene, located 16 kb downstream of the human glucocerebrosidase gene (Horowitz et al., 1989).

Type 2 Gaucher disease

Type 2 Gaucher disease is the rarest and most severe form of the disease and was first recognised as a distinct phenotype in 1927 (Tayebi et al., 1999). The frequency of classic type 2 Gaucher disease in the general population ranges from 1 in 100,000 to 1 in 500,000 births (Beutler & Grabowski, 1995). Classically type 2 Gaucher disease was considered a disease of late infancy. Patients were described as normal at birth but later displayed increasing hepatosplenomegaly, the regression of developmental milestones, and an arrest of growth, death generally occurring before patients reach two years of age. Affected individuals may be detected prenatally, often with hydrops fetalis or postnatally when they may present with a “collodion baby” phenotype. The neurologic disease in type 2 is progressive, including cranial nerve and extrapyramidal tract involvement, with death resulting from apnoea or aspiration (Stone et al., 2000). The common neurological findings are strabismus and muscular hypertonicity or spasticity with persistent retroflexion of the neck. Skin samples from patients with type 2 Gaucher disease had dense hyperkeratosis, epidermal hyperplasia, and inflammation. Ultrastructural analysis showed abnormal arrays of loosely packed lamellar body-derived sheets replacing the normal lamellar bilayer unit structures in the stratum corneum (Tayebi et al., 1999). The observation that only patients with type 2 Gaucher disease have distinct ultrastructural and lipid biochemical abnormalities may permit rapid diagnostic testing. The distinction has important therapeutic implications, since patients with type 2 Gaucher disease are less likely to benefit significantly from the enzyme replacement (Bove et al., 1995). Unfortunately there is no treatment that can reverse or halt the neurologic sequelae associated with type 2 Gaucher disease. Enzyme replacement therapy is widely used in type 1 Gaucher disease. The therapy partially treats the anaemia and organomegaly in patients with type 2 Gaucher disease and can prolong life slightly, but is not recommended, as
affected children continue to deteriorate neurologically. Attempts have been made to deliver the enzyme directly into the central nervous system by intraventricular infusions through an Omya reservoir, but neurological deterioration continued (Tayebi et al., 1999). In vitro studies with an N-alkyl derivative of the imino sugar N-butyldeoxynojirimycin (NBDNJ) demonstrated that this compound prevented the storage of glucocerebrosides in cells (Platt et al., 1994).

Lung involvement in Gaucher disease

Symptomatic lung involvement in Gaucher disease is relatively rare, being restricted to patients with severe manifestations (Goitein et al., 2001). Pulmonary involvement is not uncommon in the infantile form but is considered rare in the adult type (Amir & Ron, 1999). It has been suggested that homozygosity for the mutation L444P is associated with a major risk of developing intrinsic pulmonary involvement. In these patients primary lung disease is also likely to occur at early ages and does not seem related to the duration of the enzymatic therapy (Santamaria et al., 1998). Sporadic case reports have shown various types of lung involvement, but the spectrum of pulmonary pathology in Gaucher disease has not been described (Amir & Ron, 1999). Takahashi and coworkers reported that postmortem examination of a Japanese patient with Gaucher disease type 2 revealed massive intra-alveolar infiltration of Gaucher cells in lungs and central nervous system (Takahashi et al., 1998). Carson and coworkers reported a case of Gaucher disease with pulmonary involvement. Numerous Gaucher cells were seen in the bronchoalveolar lavage (BAL) fluid of the reported patient (Carson et al., 1994). Pulmonary pathology includes Gaucher cell infiltration of the alveolar or interstitial spaces, peribronchial or vascular infiltration, or arteriovenous shunting, most often as a complication of long-standing liver disease “hepatopulmonary syndrome” (Goitein et al., 2001). The respiratory symptoms reported in Gaucher patients with pulmonary involvement include attacks of respiratory arrest, dyspnea, cough, sputum, tachypnea, and bronchopneumonia (Amir & Ron, 1999). In the neurological form there is a greater tendency to lung infections, aspiration pneumonia is commonly the penultimate complication in the terminal stages of type 2 babies (Goitein et al., 2001). This tendency to infection has been associated with an abnormality in neutrophil migration (Aker et al., 1993). The radiological features include consolidation, pleural effusion, interstitial thickening, patchy infiltrates, and hypoplasia of the lungs (Amir & Ron, 1999). Pulmonary function abnormality can be found among type 1 Gaucher patients, despite the lack of clinical signs and symptoms. These abnormalities include alveolar-capillary diffusion abnormality, small airway obstruction with reduced expiratory flows, and reduction in lung volume (Kerem et al., 1996). Santamaria and coworkers have recommended that in Gaucher disease a comprehensive evaluation
of pulmonary disease is made as soon as possible, even in the absence of clinical symptoms. It should primarily include imaging studies of the lung (chest x-ray and high-resolution computer tomography) (CXR and HRCT) to diagnose air space and/or interstitial disease, and pulmonary function tests (PFT) to detect functional impairment. The patients should also be examined for pulmonary vascular disorders through noninvasive tests such as echocardiography to evaluate pulmonary hypertension and the administration of 100% oxygen to reveal even small amounts of intrapulmonary shunts (Santamaria et al., 1998).

Asthma

Asthma is a chronic disorder, which tends to increase in both prevalence and severity, affecting over 100 million people worldwide (Lenfant & Khaltaev, 1995). The disease affects people of all ages. Asthma was described many centuries ago as attacks due to, for example, sleeping in feather beds (Mann, 1984). Asthma is a disorder of reversible airway obstruction, associated with mucosal inflammation and increased responsiveness in the airways (Jeffery, 1999). The limited airflow in asthma is due to three major factors: oedema and thickening of the wall, mucus plugs in the airway lumen, and smooth muscle contraction (Rees & Price, 1997). The current definition of asthma states that: “asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils, and T-lymphocytes. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough particularly at night and/or in the early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli (International consensus report on diagnosis and management of asthma, 1992). Three types of asthma are usually distinguished: extrinsic (allergic), intrinsic (non-allergic), and occupational forms (Jeffery, 1999). Extrinsic asthma is IgE-mediated, atopy-associated and there is a childhood onset. Intrinsic asthma is of unknown aetiology and there is an adult onset (National Heart Lung and Blood Institute, 1992). Atopy is the strongest identified risk factor for the development of asthma (Burrows et al., 1989). There has been a lack of agreement on the definition of the term atopy. Genetically allergy is associated with IgE antibody production and atopy (Corrigan, 1998). In the present study we have used the definition of atopy proposed by an international consensus rapport. Atopy was defined as a skin reaction to one or more of the allergens with a mean diameter of ≥ 3mm and no dermatographism (Dreborg & Frew, 1993). The prevalence of allergic asthma has increased decades earlier in Western Europe compared to Eastern Europe. This is
probably due to changes in lifestyle that had already occurred rather than to air pollution (Matricardi, 2001). Genetic predisposition and environmental factors interact to produce asthma. The multigenic nature of asthma has hampered progress in identifying asthma-susceptibility genes. The Th-2 cytokine gene IL13 is one of the candidate genes. ADAM33 was also identified as an asthma-susceptibility gene. ADAM33 seems to function in airway remodeling. The chromosome13q14 gene PHF11 was recently identified as a locus for IgE levels in asthma (Wills-Karp & Ewart, 2004).

Epithelial damage in asthma

Light and electron microscopic studies of bronchial biopsies in asthma have added valuable information to that obtained from studies of lungs taken at necropsy. In particular, mucosal biopsies taken during bronchoscopy have highlighted the early damage to surface epithelium in atopic asthma, even of mild severity (Jeffery et al., 1992; Knight & Holgate, 2003). In severe asthma bronchial epithelial cells are damaged and detached, which may be related to the bronchial hyperresponsiveness that characterizes asthma (Laitinen et al., 1985; Lozewicz et al., 1990; Benayoun et al., 2001, 2003). Laitinen and coworkers (1985) observed increased detachment of the bronchial epithelium in more aggravated asthma cases, leaving only a layer of basal or reserve cells on the basal lamina (Laitinen & Laitinen, 1994a). In bronchial biopsy specimens from children with asthma, the ciliated epithelial cells showed loss of cilia and overactive fibroblasts, and the inflammation was rich in lymphocytes rather than eosinophils (Cokugras et al., 2001). The relationship between epithelial damage and airway responsiveness has been studied by several workers (Ohashi et al., 1992). Epithelial shedding is characteristic of bronchial asthma. Bronchial washings reveal that epithelial cells are shed in clusters composed entirely of columnar cells, and asthma patients have an increased number of epithelial cell clumps (creola bodies) in their sputum (Woltmann et al., 1999). According to electron microscopy the basal cells remain attached to the basal lamina. Columnar epithelial cells do not form hemidesmosome attachments with the basal lamina, which may explain this observation of columnar cell sloughing (Montefort et al., 1992; Montefort et al., 1993). Some studies have shown that shedding of the columnar epithelial cells starts in the middle of the epithelium, where the columnar epithelial cells are still attached to each other at the luminal side, but separated from the basal cells by oedema fluid (Laitinen & Laitinen, 1994a). Widening of the intercellular space may contribute to the shedding process. This widening of the intercellular space has been reported by others (Ohashi et al., 1992). The mechanisms underlying the epithelial cell derangement are likely to be complex due to the complex nature of epithelial cell attachment to the basement membrane (Venaille et al., 1989). The mechanism of epithelial shedding is not clear. In asthma there may be a primary defect in the
epithelium, which leads it to respond abnormally to various stimuli and also prevents it from proceeding towards a normal repair process (Holgate, 2000). It has been hypothesized that airway hyperreactivity in asthma may be related to epithelial desquamation through mechanisms involving loss of epithelium-derived relaxing factor (Fahy, 2001). It has been shown that failure of epithelial cells to adhere adequately to the basement membrane can result in more extensive penetration of inhaled antigens followed by mediator response (Beasley et al., 1993). Certain allergens, for example the house dust mite Der P 1 could contribute to sensitisation and allergic responses by degrading the function of the airway epithelial barrier (Wan et al., 2000). It is possible that damage to tight junctions and influx of ASL or toxic agents through widened intercellular spaces can cause further disintegration of the epithelium. Furthermore, leukocytes and their granules can gather within the widened intercellular spaces between the basal cells and superficial columnar cells thereby aggravating the situation (Roche et al., 1993). Epithelial integrity may be important in preventing the penetration of inflammatory cells. Adhesion molecules and cell contacts play a crucial role in the maintenance of this integrity and it has been suggested that tight junctions and/or desmosomes and hemidesmosomes may be affected in asthmatics (Laitinen & Laitinen, 1994b).

The bronchial epithelium in asthma is more susceptible to damage than normal epithelium. Mullings and coworkers have recently described constitutive activation of signal transducer and activator of transcription (STAT)-6 in asthmatic epithelium compared to epithelial cells from healthy controls. STAT-6 is the signal transducer used by IL-4 and IL-13 receptors (Mullings et al., 2001). Expression of EGFR (epidermal growth factor receptor) is markedly upregulated in the epithelium of adult asthmatics, especially where basal cells have lost their columnar cell attachments (Knight & Holgate, 2003). Bucchieri and coworkers have also shown that asthmatic epithelial cells are more susceptible than normal cells to oxidant-induced apoptosis (Bucchieri et al., 2002). However, the question as to whether the differences observed in asthmatic epithelium are a cause of or secondary to, the development of the disease remains unanswered.

Airway Remodeling

Remodeling is a critical aspect of wound repair in all organs, representing a dynamic process in reaction to an inflammatory insult. Chronic inflammation is always followed by healing, beginning very early and resulting in the end in repair (Bousquet et al., 1995). Remodeling results in thickening of the airway wall, including subepithelial collagen deposition, and submucosal collagen deposition. When the epithelium is exposed to a variety of different exogenous factors such as allergens, air pollution or viruses, or endogenous
factors such as proteolytic enzymes, the epithelium is unable to respond ade-
quately by reconstructing itself (Holgate, 1998). As a consequence of this
impaired response to stress, a variety of molecules such as growth factors
and cytokines, are generated to create repair by secondary processes (Hol-
gate et al., 1999).

Thickening of the airway wall
Earlier studies have observed that the basement membrane was thickened in
asthmatics (Cokugras et al., 2001; Laitinen et al., 1997). Airway wall thick-
ness is increased 50-300% in cases of fatal asthma and 10-100% in cases of
nonfatal asthma (James, 1997). It is well known that asthmatic airways are
characterised by subepithelial fibrosis (Hoshino et al., 1998; Agarwal et al.,
2003). It is thought that there is an abnormal communication between the
myofibroblasts that deposit collagen in the epithelial sub-basement mem-
brane, and the epithelium (Holgate et al., 2000). However, there is also in-
creased deposition of collagen in the deeper layers of the submucosa (Wilson
& Li, 1997). The number of myofibroblasts is increased in asthma and corre-
lates with the extent of collagen deposition (Brewster et al., 1990). A finding
that also contributes to airway wall thickening in asthmatics, besides an in-
crease in smooth muscle, glandular hypertrophy, oedema, inflammatory cell
infiltration, and connective tissue deposition, is cell proliferation (Elias,
2000). This proliferation is assumed to be due to mediators generated from
the epithelium such as epidermal growth factor (EGF) (Holgate, 2000).

Infiltration of inflammatory cells
The number of eosinophils, mast cells, macrophages and activated T lym-
phocytes is increased in asthma (Amin et al., 2000; Ohashi et al., 1992).
Ohashi and coworkers reported that with increased density of eosinophils
infiltrated in the bronchial mucosa, both the incidence of opening of tight
junctions of epithelial ciliary cells and the degree of widening of intercellular
spaces in the epithelium increased significantly, suggesting that eosinophils
are related to damage of the bronchial epithelium (Ohashi et al., 1992).

Goblet cell hyperplasia
Among the changes that occur in asthma is the marked hyperplasia of the
goblet cells with the development of intra-epithelial mucus glands (Konra-
dova et al., 1985; Ordonez et al., 2001).
Cell death

Removal of cells that are no longer of use is as important as cell proliferation during airway repair and remodeling. Necrosis is the death of cells through injury or disease. On the other hand, apoptosis is the process by which a cell actively commits suicide. Cell death can be triggered by the disruption of cell-matrix contacts (Gailit & Clark, 1994). The body produces both pro- and antiapoptotic proteins and the sensitivity of the cells to them varies. The immune system triggers viral infected cells, or cells that show mutations (potential tumor cells) to commit apoptosis. Different types of cellular stress caused by chemicals, radiation, virus or oxidation induces apoptosis (www.sgul.ac.uk). When a cell receives a signal to actively commit suicide, or is being stimulated to do so due to the absence of survival signals, a chain of distinct biochemical and morphological changes occurs. In most cases a family of proteases (caspases) is activated (Alberts et al., 2002).

Inflammatory response in asthma

Symptoms of chronic asthma are associated with the recruitment of inflammatory cells, predominantly eosinophils, mast cells, lymphocytes and neutrophils and subsequent release of their mediators (histamine, leukotriens, and cytokines). Cytokines are produced by human Th-1 and Th-2 lymphocytes. Th-1 lymphocytes produce interferon gamma (INF-γ), IL-2, and TNF-α, whereas Th-2 lymphocytes produce IL-4, IL-5, IL-9, IL-10, and IL-13 (Feghali & Wright, 1997).

TNF-α and INF-γ

TNF-α is produced by macrophages and natural killer cells and is associated with inflammation, septic shock and wasting during chronic disease (Vilcek & Lee, 1991). In the acute situation, local production of TNF-α is beneficial. It increases the expression of adhesion molecules on the vascular endothelium to allow immune cells to traffic to sites of tissue damage and infection (Gamble et al., 1985; Barbara et al., 1996). INF-γ is secreted by T-lymphocytes and is known to inhibit cell proliferation and induce cell death (De Saint Jean et al., 1999). The permeability of the epithelial layer can be modulated by INF-γ (Adams et al., 1993). TNF-α and INF-γ maybe involved in the upregulation of ICAM-1 expression (Dustin et al., 1986; Springer, 1990). Factors produced by inflammatory cells, such as proinflammatory cytokines may play a role in reducing the expression of desmosomes (Kampf et al., 1999). In a study by Carayol and co-workers TNF-α induced a decrease in E-cadherin and β-catenin expression in human bronchial epithelial
cells, and this effect was inhibited by the addition of dexamethasone (Carayol et al., 2002a).

Leukotriens
Cysteinyl leukotriens (cys-LT) (LTC4, LTD4, LTE4) are important inflammatory mediators in the pathophysiology of asthma. These compounds are synthesized in vivo from arachidonic acid through the 5-lipoxygenase pathway and are produced by inflammatory cells such as eosinophils, mast cells and basophils. Cys-LTs are potent bronchoconstrictors, promote mucus production, increase the permeability of veins and can contribute to the airway remodelling process (Bronsky et al., 1997). Cys-LTs also induce human eosinophil locomotion and adhesion molecule expression (Fregonese et al., 2002). These compounds exert their effects through binding to receptors, and inhibition of these receptors has been shown to be effective in the treatment of asthma patients (Leick-Maldonado et al., 2004).

Treatment of asthma
Bronchodilators and anti-inflammatory drugs are treatments used to ease the inflammatory symptoms in asthma.

Corticosteroids
Corticosteroids have been recommended for the treatment of asthma because these substances are considered to improve lung function, prevent inflammation and airway remodelling and promote the repair of the epithelial damage that occurs in the airways of asthma patients (Barnes et al., 1998). Corticosteroids have, however, many potentially negative systemic effects, both in children and adults. Therefore, inhaled corticosteroids have been developed since they have a very low systemic absorption (Gupta et al., 2004). Some studies on cell lines and on animal models have shown that corticosteroids can damage the airway epithelium and stimulate apoptosis of epithelial cells in asthma patients (Dorscheid et al., 2001; Dorscheid et al., 2003; White & Dorscheid, 2002; Allen et al., 2003), but other studies have found positive effects of corticosteroids, e.g., they prevent the thickening of the basal membrane on which the airway epithelium rests (Shiba et al., 2002) and increase the efficiency of β-adrenergic agonists (Aksoy et al., 2002). Suppression of epithelial ICAM expression by glucocorticoids may contribute to their anti-inflammatory effects (Atsuta et al., 1999). Some studies have shown that corticosteroids have an apoptotic effect on eosinophils, thymocytes, and lymphocytes, which may contribute to the mechanism of their anti-inflammatory effect (Avery & Woolfrey, 2003; Druilhe et al., 2003). The
question whether corticosteroids should be used in the treatment of children in the present extent has recently caused controversies (Bush et al., 2003).

**Montelukast**

Montelukast is a potent leukotriene receptor antagonist (LTRA) that blocks the action of the cys-LTs (Migoya et al., 2004). Cys-LTs are known to cause bronchial obstruction, mucosal oedema, and infiltration of eosinophilic granulocytes and increase bronchial responsiveness (Bisgaard, 2003). Montelukast causes a decrease in serum concentrations of inflammatory markers: IL4, soluble ICAM-1, and eosinophilic cationic protein (ECP) levels in children with asthma (Stelmach et al., 2002). In asthmatic subjects neurokinin A (NKA) induced bronchoconstriction is indirectly caused by the release of LTs, and this mechanism could explain some of the antiasthmatic and anti-inflammatory effects of LTRA (Crimi et al., 2003). Biological effects of montelukast delineate a complex picture of gene activation and repression, probably induced by cys-LT receptor blockade, which leads to apoptosis of activated T cells. Inducing apoptosis as a therapeutic anti-inflammatory goal to reduce excessive cell population appears fundamental in the treatment of asthma (Spinozzi et al., 2004). Montelukast has demonstrated efficacy and tolerability in the treatment of chronic asthma in patients 2 years of age and older (Knorr et al., 2001). Montelukast may be a therapeutic agent in pathological conditions involving fibrotic and remodelling processes (Fireman et al., 2004).

**Primary ciliary dyskinesia**

PCD is a congenital disease, which is usually due to a defect in ciliary dynein, but in some cases it is due to mutations in other important ciliary proteins. Several mutations have been implicated in PCD. PCD symptoms are chronic airway disease (bronchitis, rhinitis, and otitis), often infertility in men and situs inversus in 50% of patients (Noone et al., 2004). Female subfertility and tendency to ectopic pregnancy have also been suggested (Eley et al., 2005). Unlike cystic fibrosis, the diagnosis of PCD is difficult, but recently, progress has been made in elucidating the genetics of the disease. Several genes that encode for dynein arms (DNAI1, DNAH5, DNAH9, and DNAH11) have been linked to recessive PCD (Fliegauf et al., 2005; Van's Gravesande & Omran, 2005) and several other genes have been named as candidate genes for other variants of PCD (Pennarun et al., 2002; Zariwala et al., 2004). The disease thus is genetically heterogeneous.

Kartagener described a triad of abnormalities (sinusitis, bronchiectasis, and situs inversus) in four patients and first recognized a clinical syndrome,
which was subsequently named after him (Kartagener, 1933). Afzelius et al. described the association of sinusitis, bronchiecstasy, and situs inversus with an absence of dynein arms in the tails of the immotile spermatozoa in infertile men (Afzelius et al., 1975). The term “immotile cilia syndrome” was introduced by Eliasson et al., to describe all congenital ciliary defects resulting in impaired mucociliary clearance and male infertility (Eliasson et al., 1977). Soon it was determined that lack of motility was not absolute. In some patients, ciliary movement was observed; nevertheless, it was not adequate for normal mucociliary clearance. Therefore, the syndrome name was changed to “dyskinetic cilia syndrome” (Rossman et al., 1980).

The suspicion of PCD can arise in patients with chronic symptoms in the upper or lower respiratory tracts where no other diagnosis is evident. Chronic inflammation of the airways can, however, give rise to acquired ciliary immotility, so-called SCD (Sleigh, 1981). Patients with SCD may have respiratory symptoms that cannot be distinguished from those of PCD.

### Diagnosis of PCD

Diagnosis of PCD is often delayed despite the presence of typical symptoms early in life (Coren et al., 2002). Early diagnosis may prevent the development of bronchiecstasy and the subsequent decline in lung function (Bush & O’Callaghan, 2002). Suspicion of the diagnosis is based on a good clinical history examination, and unspecific methods such as radiography and pulmonary function test.

Specific methods include screening tests, such as the saccharin test and nasal NO measurement. The saccharin test is very difficult to perform properly in children (Afzelius, 2004). A more serious disadvantage of the widely used saccharine test is that the solubility of saccharine permits it to diffuse into the periciliary layer of the mucus, where transport is slow and irregular. However, a negative result is not diagnostic of PCD (Armengot Carceller et al., 2005). The measurement of nasal NO appears to be a useful tool to screen children for PCD and exclude it in those with high nasal NO levels; nasal NO levels above 105 ppb appeared to exclude PCD with 100% certainty (Corbelli et al., 2004).

Electron microscopy or analysis of ciliary beat frequency (CBF) are commonly used, and require that a biopsy of the respiratory mucosa be carried out. When tracheal biopsies are performed, there is the additional expense of bronchoscopy, as well as a higher potential morbidity (Friedman et al., 2000). Biopsies of nasal mucosa may be performed by means of a curette or with the brush technique. Nasal biopsy is preferred over bronchial or tracheal biopsies (Ahmad & Drake-Lee, 2003) since it is simpler, less invasive
and could be performed in an outpatient setting. However, nasal biopsy has one disadvantage; since the nasal mucosa is the site of frequent viral and bacterial infections, functional and structural alterations in the cilia secondary to infectious processes may be observed.

Several methods have been developed to measure ciliary function. These methods are high-speed cinematography, laser light spectroscopy, photoelectric measurements, and stroboscopy. Although these techniques are fairly established, they require complicated equipment, extensive training, and are available in very few centers. In addition, they do not provide any clues to the actual underlying cause for an absence or slowing of mucociliary clearance. In addition, physical factors such as changes in posture, coughing, sneezing, and food intake all could significantly affect the results of these studies (Tsang et al., 2000). Light microscopy evaluation of ciliary motility does not appear to be a reliable screening test for ciliary dyskinesia because it does not quantify ciliary beat activity, which is a criterion for deranged ciliary motion (Santamaria et al., 1999).

The analysis of CBF alone may miss the diagnosis in patients with ciliary transposition, because beat frequency is often normal in this ultrastructural defect since it does not affect all cilia (Stannard et al., 2004). The most commonly used techniques (the modified photodiode or photomultiplier method) to measure CBF use an indirect method and do not provide information on ciliary beat pattern. New high-resolution digital high speed video imaging has allowed the precise beat pattern of cilia to be viewed. Specific ultrastructural defects responsible for PCD result in specific abnormalities in beat pattern and beat frequency (Chilvers et al., 2003).

The gold standard for diagnosis of PCD is by ultrastructural evaluation of cilia by TEM. Whereas the absence of outer dynein arms can be easily distinguished, the absence of inner dynein arms is difficult to confirm because of their low contrast in the electron microscope. To improve inner dynein arm visualization, computer-assisted analysis of electron microscopic micrographs was developed, as well as the use of tannic acid staining (Escudier et al., 2002). Computer-assisted analysis of radial symmetry has also been developed (Carson et al., 2000). Although the diagnosis of PCD is most frequently based on a dynein deficiency found in TEM, there are many differential diagnostic problems both ultrastructurally and functionally and in many cases even no ultrastructural abnormality is found (Willems & Jorissen, 2004).

A more sensitive and specific method for the diagnosis of PCD is ciliary analysis both structurally and functionally, after ciliogenesis in culture. The cilia formed in vitro are completely normal in cases of SCD. There are a
number of limitations and disadvantages to the cell culture system. First, there is need of a biopsy to start the culture, thereby inducing some morbidity. Secondly, the cell culture procedure may fail, due to insufficient epithelial cells or infection. Thirdly, special equipment, experience and expertise are needed. Fourthly, the total procedure takes 6 weeks (Jorissen et al., 2000a,b,c; Grella et al., 2001).

At present, the most common method to diagnose PCD is by electron microscopy of ciliated cells from the nasal epithelium.
AIMS

- To assist in the clinical diagnosis of a paediatric patient with severe, chronic respiratory symptoms (paper I).

- To obtain more information on the epithelial cell attachments in the airways with special focus on desmosomes and hemidesmosomes. There have been very few studies on desmosomes and their connection with asthma. Clearly then, epithelial cell attachment/detachment mechanisms in health and disease need to be studied (paper II).

- To test the reliability of the use of rats as an animal model for the study of diseases affecting the airway epithelium (paper II).

- To investigate the morphological difference between patients with PCD and patients with SCD, in order to develop firm diagnostic criteria to distinguish PCD and SCD (Paper III).

- To investigate and compare the effect of corticosteroids and a leukotriene receptor antagonist on apoptosis and necrosis of airway epithelial cells, on the expression of desmosomes in airway epithelial cells and on the expression of cell adhesion molecules in airway epithelial cells and endothelial cells (paper IV).
MATERIALS AND METHODS

Case report of a paediatric patient with respiratory symptoms

The boy was born after an uneventful pregnancy at a normal delivery as the 2nd child to a 46-year-old woman. His weight and length at birth were 2950 g and 46 cm respectively. Both parents were healthy and of Thai descent. There was no family history of neurological, skeletal or mental disorders. There were no neonatal complications and he was discharged in good condition. At three months of age the child started to develop feeding and respiratory difficulties. The boy’s motor and mental development continued to be normal for age but he had a tendency to hyperextend his neck, especially when his respiratory problems increased. His muscle tone was, however, normal. The gag reflex was present but there was some pooling of secretions and poor swallowing. At this time no hepatosplenomegaly was found.

At 5½ months of age the boy was admitted to the hospital. Laryngoscopy showed a tendency to laryngomalacia and hypotonia of the pharyngeal muscles. A bronchoscopy including a tracheal biopsy and a nasal brush biopsy was performed at 6½ months of age. TEM of the tracheal epithelium indicated a lysosomal storage disease and the nasal brush biopsy indicated secondary ciliary dyskinesia. An X-ray of the oesophagus revealed dilatation and abnormal swallowing, and a 24-hr pH measurement showed a pathological gastro-oesophageal reflux. As the boy still did not have hepatosplenomegaly he was suspected to have Sandifer’s syndrome (Mandel et al., 1989), and was initially treated conservatively, without any improvement during two months. At 7½ months a clear retardation of motor development was observed, but the boy’s mental development was still normal for age.

At 8½ months surgical correction of the gastro-oesophageal reflux and a gastrostomy was performed. During the operation a hepatosplenomegaly was discovered. Neurological examination now revealed a convergent strabismus, a constant hyperextension of the neck and exaggerated difficulties when swallowing, all regarded as signs of bulbar dysfunction.
At 9½ months, liver, spleen and bone marrow biopsies were performed. The diagnosis of Gaucher disease was finally made on clinical signs and confirmed by low beta-glucocerebrosidase activity. Histopathological studies of the spleen and bone marrow showed Gaucher cells. According to the clinical findings and age at start the patient was classified as type 2 Gaucher disease. Genetic analysis revealed the genotype as L444P/unknown.

Glucocerebrosidase replacement (Cerezyme®, 175 units twice weekly) was started, without any improvement, and the boy deteriorated and died of respiratory failure at an age of 11 months. (Paper I)

Bronchial biopsies
Bronchial biopsy specimens were taken from five healthy adults and five patients with atopic asthma. The study was conducted in accordance with the declaration of Helsinki and was approved by the Ethics Committee at the Faculty of Medicine at the University of Uppsala.

Controls
The controls were healthy persons who responded to a request for volunteers. No one had asthmatic symptoms and all showed a negative skin prick test. (Paper II)

Atopic asthmatics
All five patients had a clinical diagnosis of asthma, current asthma symptoms and increased responsiveness to inhaled methacholine, defined by a provocation dose of methacholine causing > 20% fall in FEV1 (PC20) <32 mg. All patients had a positive skin prick test (> 3mm) for at least one common allergen tested: birch, timothy grass, mugwort, cat, dog, horse, house dust mite, cladosporium, and Alternaria. All but one of the patients were on regular treatment with inhaled glucocorticosteroids (budesonide 200-800 µg/day) and inhaled beta agonists as needed. These patients were being followed as outpatients at the Department of Respiratory Medicine and Allergology at Uppsala Academic Hospital. None of the patients were smokers and they had been free from respiratory infections for at least six weeks prior to bronchoscopy and none had a history of cardiovascular disease. Before the participants were included in the study, they had answered a questionnaire about airway symptoms during the last twelve months. The questionnaire was based on the European Community Respiratory Health Survey Questionnaire (Burney et al., 1994). The methods for the allergic test, spi-
Symptom score and peak flow variability

Records of symptoms and peak flow variabilities were kept during a 17 day period starting on the day of the metacholine challenge. The peak expiratory flow rate (the best of three measurements) was recorded four times daily with a Mini-Write Peak Flow Meter (Clement Clare, London, UK). Minimum requirements were at least two recordings a day, one of which was in the morning. Peak flow variability was calculated by dividing the difference between the highest and lowest daily PEF reading by the daily mean PEF value. The results were expressed as the daily percentage of variability (vPEF%). In the symptom score, the subjects stated on awakening whether they had had: breathing difficulties during the previous night, wheezing in the chest, attacks of breathlessness or attacks of coughing during the previous 24 hours. Each affirmative answer was given a score of one and a symptom score was calculated (Ludviksdottir et al., 1999). (Paper II)

Bronchoscopy

The patients were given 10 mg of diazepam orally and 0.5 mg of atropine subcutaneously 30 minutes before the investigation. The upper airways were anaesthetized with lidocaine hydrochloride. Using a flexible fibre bronchoscope (Olympus P 20D) with a FB 15C 2.0mm forceps (Olympus), two biopsies were taken from the right lung from the upper lobe bronchus immediately after the division from the main bronchus. The specimens were examined immediately by a light microscope to ensure the presence of a complete mucosa and fixed as described below. The patients were instructed to take their regular asthma spray on the morning of the bronchoscopy. (Paper II)

Rats

Three adult male Sprague Dawley rats (weight about 300 grams) were purchased from B&K Universal (Sollentuna, Sweden) and housed in a conventional animal care facility at the Biomedical Center, Uppsala University one week before experimentation. The Regional Committee on Animal Experimentation, Uppsala, approved the protocol. All rats were anesthetized intraperitoneally by sodium pentobarbital injection, and were dissected when reflexes could no longer be elicited. The tracheas were removed from the rats, and tissue taken from the middle part was fixed for electron microscopy. (Paper II)
Nasal brush biopsies

A retrospective study was made of all cases referred for electron microscopical investigation by clinics at Uppsala University Hospital between January 2002 and January 2005. The patient group consisted of 16 patients with PCD and 19 patients with SCD. The diagnosis of PCD or SCD was made based on the electron microscopical investigation and the clinical examination of the patient. To determine the ciliary orientation, two equally large subgroups from these populations were chosen, consisting of 15 PCD patients and 15 SCD patients. In addition, 7 healthy controls were included in the study. Most of the cases had signs and symptoms suggestive of PCD (upper respiratory problems, blocked or runny nose, sinusitis, and otitis). Since this was a retrospective study, no treatment was discontinued at the time. Thus, most of the patients were on bronchodilators, mucolytics, antihistamine, and inhaled corticosteroids and physiological solutions. Nine PCD patients and 7 SCD patients were on antibiotics during the time the samples were taken.

Three patients had no clinical symptoms, 2 were investigated because a first degree relative had PCD, and one neonate was investigated due to the finding of dextrocardia. Spirometry was not tested in young patients and controls.

The nasal epithelial cells were obtained by a brush biopsy of the middle concha (inferior turbinate), with the exception of one sample that was obtained by bronchoscopy from the main bronchus. (Paper III)

Cell lines

16HBE cells (Cozens et al., 1994) were a kind gift of Dr D. Gruenert (San Francisco, CA, USA). These cells are normal airway epithelial cells (columnar cell line). 16HBE cells were cultured in Eagle’s minimal essential medium (EMEM; SVA, Uppsala, Sweden) with 10% (v/v) fetal calf serum (Gibco; Invitrogen, Carlsbad, CA, U.S.A.) and 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were grown to confluence in 25 cm² culture flasks (Sarstedt, Nümbrecht, Germany) with a positively charged surface.

Calu-3 cells (ATCC, Manassas, VA, USA), a cell line originating from airway submucosal glands was cultured in the same medium, to which 1µM sodium pyruvate and 1% (v/v) non-essential amino acids (Sigma-Aldrich, St. Louis, MO, USA) had been added.

HUVEC (human umbilical vein cells) (PromoCell, Heidelberg, Germany) were grown in endothelial cell growth medium with 2% (v/v) fetal calf se-
rum, 0.4% (v/v) ECGS (endothelial cells growth medium with heparin), 0.1
ng/ml human recombinant EGF, 1.0 ng bFGF (human recombinant basic
fibroblast growth factor) and 1.0 µg/ml hydrocortisone (Promocell). The
HUVEC were used between the first and tenth passage.

For the experiments on apoptosis and necrosis, the cells were grown to
confluence on 24- or 96-hole plates (Sarstedt), in a cell culture incubator at
37°C, 5% CO₂ and 95% humidity, with a change of medium every second
day. 16HBE cells took two-three days to reach confluence, Calu-3 cells 3-4
days and HUVEC up to 6 days. (Paper IV)

Induction of cell cultures
The corticosteroids dexamethasone, beclomethasone and budesonide were
all from Sigma (St. Louis, MO, USA). Montelukast (a leukotrien receptor
antagonist) was a generous gift from Merck, Sharp & Dohme (Sollentuna,
Sweden). The active substances were dissolved in 95% ethanol (corticostero-
dids) or in distilled water (montelukast), but the final ethanol concentration
did not exceed 0.1% (v/v) for 16HBE cells or 0.005% (v/v) for Calu-3 cells.
To the controls and the cultures exposed to montelukast, ethanol was added
to obtain the same concentration as was present with the corticosteroids.

The pan-caspase inhibitor Z-VAD-fmk was from Calbiochem (La Jolla,
CA, USA). TNF-α and IFN-γ were from BioSite (San Diego, CA, USA).
(Paper IV)

Transmission electron microscopy (TEM)
16HBE cells grown on Petri dishes, bronchial, tracheal and nasal brush bi-
opsy tissues, as well as pieces of rat trachea were fixed in 2-2.5% glutaralde-
hyde in 0.1 M sodium cacodylate buffer for one day. After being washed in
cacodylate buffer, the specimens were post-fixed in 1% OsO₄ in cacodylate
buffer for 60 to 120 minutes. A second wash in buffer was followed by de-
hydration in a graded series of ethanol and propylene oxide, and were finally
embedded in Agar 100 epoxy Resin (Agar Scientific, Stanford, U.K.). Ultra-
thin sections were cut, contrasted with uranyl acetate/ lead citrate, and exam-
ined in a Hitachi H 7100 TEM (Hitachi, Tokyo, Japan) at 75 kV. (Paper I,
II, III, IV)

Microscopic evaluation of bronchial biopsy sections
The TEM image was transferred via a digital camera to a Synopsis (Cam-
bridge, UK) Synapsee frame capture and image analysis system. The meas-
measurements were carried out at an original magnification of 1000X. To determine the contact area between columnar cells and basal cells and the basal lamina, in each human specimen measurements were taken from two non-adjacent areas randomly chosen from areas with an intact epithelium. For the rat specimens measurements were taken from three separate areas in each rat. An average total length of 119 µm was studied for each rat.

The length of the basement membrane was determined for every area. An average of 81 µm of basal lamina for every human control sample, and of 99 µm for every asthma patient was used. The number of nucleated basal cells was counted for every area and expressed per 100 µm of basal lamina. The height of the columnar cells was determined by measuring the height of a randomly chosen cell in the center of each area. The height of the epithelium (i.e., columnar plus basal cells) was also determined in the center of each area. Cilia were not included in these measurements. Columnar and basal cell surfaces in contact with the basal lamina were determined by tracing the length of their attachment to the basal lamina. The data are presented as columnar or basal cell contacts per 100 µm of basal lamina. The columnar cell surface in contact with basal cells was also determined by tracing, and the data were normalized with regard to the length of the basal lamina. (Paper II)

Quantitative analysis of ciliary ultrastructure

The specimens of the patients and healthy controls were examined in a double-blind manner, i.e., the microscope operators and those evaluating the electron micrographs were not aware of the clinical diagnosis. The ciliated epithelium was assessed for both epithelial and ultrastructural changes. Epithelial integrity was assessed quantitatively or semiquantitatively, by examining cell type at an original magnification of 3000x to 7000x. For each sample, the number of ciliated cells, mucus (goblet) cells, cells with few cilia, non-ciliated cells (cylindrical cells with microvilli only), and dead cells were counted and expressed as a percentage of the total number of cells in a section. Cytoplasmic blebbing and mitochondrial damage were among the main parameters for distinguishing dead cells. Polymorphonuclear cells, lymphocytes, and neutrophils were noted as inflammation markers.

Damage to individual cilia was evaluated by examining the ciliary ultrastructure for dynein arm, microtubular defects, and membrane defects. Magnifications of 40,000x or 50,000x were used to assess the detailed axonemal structure, including the dynein arms, whereas the magnification of 20,000x was most useful for evaluation of secondary abnormalities. Electron microscopic images were photographed for blind analysis later. An average of 4 photos was taken per sample. Care was taken not to evaluate cilia close to
the cell membrane and not too close to the ciliary tip. Cross-sections through cilia were selected in which the cell membrane appeared as a trilaminar structure, indicating a “true” cross-section.

For quantitative analysis of the number of inner/outer dynein arms per cilium, 10 cilia were evaluated per sample and the mean for every sample was calculated. In order to determine the minimum number of cross-sections that needs to be measured to establish a diagnosis, 30 cross-sections were measured in randomly chosen PCD and SCD patients. The means of inner and outer dynein arms after measurements on 1 to 30 cilia were calculated.

The biopsies were characterized morphologically, by evaluating 50 cilia in each biopsy and determining the percentage of samples that showed cilia with membrane abnormalities (missing membrane; membrane blebs), missing peripheral or central microtubules, supernumerary microtubules, dislocated peripheral microtubules, and “compound” cilia (two or more cilia surrounded by a single membrane).

Alignment of individual cilia within a cell was assessed by measuring ciliary orientation (COR). Electron micrographs of cross-sections of ciliary shafts from 15 PCD patients and 15 SCD patients taken at a magnification of x30,000 were transferred into the digital form and processed with the image analysis software Leica IM 4.0 (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK).

COR was measured only in ciliary cross-sections in which the two central microtubules could be seen. An average number of 15 cilia per patient were measured. A line was drawn through the central microtubule pair of each cilium. The angle between this line and a reference (vertical) line was measured for all cilia seen on one micrograph; this angle had to be acute. The angle is defined by sides that are directed upwards. Measuring COR angles in this manner, only acute angles between -90° (to the left of the vertical axis) and +90° (to the right of the vertical axis) can be obtained. In some cases, the angle of COR was equal to 90°. In these cases, the sign (- or +) of this angle depended on the other COR angles on the particular micrograph. If the majority of angles were negative, this angle was regarded as a right negative angle. If the majority of angles were positive, this angle was regarded as a right positive angle. In each case, the standard deviation (SD) of COR angles was calculated. This represented an index of COR for particular patient. For each group, a mean±SD value was obtained from all the SD values in this group, which represented an index of COR for particular group (PCD or SCD).
The angles of PCD and SCD patients were divided into degree intervals from -50 to 50 degrees, and presented as the relative number of cilia per interval, in order to show the distribution of ciliary orientation. (Paper III)

Microscopic evaluation of cultured cells
16HBE cells treated with TNF-α, INF-γ, dexamethasone, beclomethasone and budesonide were prepared for TEM. Electron micrographs were transferred via a digital camera to a Synopsis (Cambridge, UK) computer system with a Synaps-MAPP (Les Ulis, France) frame grabber. A total of 100 µm lateral cell membrane per sample was measured, and the relative area occupied by desmosomes was determined. (Paper IV)

Detection of apoptosis and necrosis
The proportion of apoptotic or necrotic cells was determined by the detection of propidium iodide (Sigma) with a FACS Calibur fluorescence-activated cell sorter (FACS) (Becton-Dickinson, Franklin Lakes, NJ, USA). A solution of 2 mg/ml propidium iodide in distilled water was added to the sample (10 µl per ml sample). The sample was incubated with the propidium iodide at 37°C and 5% CO₂ for 10 min. The supernatant was then transferred to Eppendorf tubes. The cells were then washed with 300 µl Hank’s solution (Sigma). To each well, 200 µl trypsin solution (2.5 g/l trypsin and 1.86 g/l EDTA) was added and the incubation lasted for 10 minutes at 37°C and 5% CO₂. The supernatant was then transferred to Eppendorf tubes and centrifuged for 2 min with an Eppendorf (Hamburg, Germany) 5414 centrifuge at maximal speed. 900 µl of the supernatant was drained carefully, which left the cells in a pellet at the bottom of the tube. 900 µl new growth medium was added and the pellet was resuspended. The cell suspension was then transferred to Falcon tubes and fluorescence was detected at 610 nm. The number of cells was determined with Cellquest software (BD Biosciences, San José, CA, USA) and 5000 cells were counted in each sample. (Paper IV)
Immunocytochemical studies (ICAM expression)

After incubation, the cells were fixed in methanol for 3 min at −20°C, rinsed with Tris-buffered saline (TBS), pH 7.6 for 5 min and then blocked with 10% normal human serum (NHS) (Sigma) in TBS for 15 min. Following primary incubation with monoclonal anti-ICAM-1 (R&D Systems, Abingdon, Oxon, U.K.) at a dilution of 1:100 in TBS for 1 h at 37°C, the cells were rinsed twice with TBS. The cells were again blocked with 10% NHS for 15 min and incubated with a FITC (Fluorescein isothiocyanate)-conjugated secondary antibody (Dako, Glostrup, Denmark) at a dilution of 1:40 for 1 h. After rinsing with TBS the slides were mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA) mounting medium and examined with a Leica DMR fluorescent microscope (Leica, Wetzlar, Germany) (Relova et al., 2005). (Paper IV)

Statistics

Statistical analysis was performed using the non-parametric Mann-Whitney test, when the number of samples was low (Paper II). When two groups were compared, an independent Student’s t-test was used; Welch’s correction was applied if the variances were significantly different (Paper III, IV). Significance was attributed to probability values <0.05.
RESULTS

Paper I: Airway biopsies of a patient with type 2 Gaucher disease

The nasal biopsies showed a normal ciliated epithelium, although the orientation of the basal feet was sometimes irregular suggesting secondary ciliary dyskinesia. The cilia had a normal structure and showed the presence of dynein arms. The columnar cells of the bronchial biopsy had few cilia. These cells showed accumulated lamellar bodies, presumably secondary lysosomes. Some of these were filled with somewhat flattened lamellar material, whereas others were smaller, with more electron dense concentric lamellae. Intermediate forms were also observed, indicating the transition of one of the forms into the other. In many cells, condensed mitochondria were observed, an unspecific sign of suboptimal metabolism.

Paper II: Attachment of columnar airway epithelial cells in asthma

Rat trachea

The rat airway epithelium was less high than the human airway epithelium (21 µm vs. 40 µm), and the basal cells were fewer in number and smaller in size compared to the corresponding cells in humans. The percentage of basal lamina covered by columnar cells was much higher in the rats (36% vs. 5%). At higher magnifications desmosome attachments between columnar and basal cells, as well as between basal cells were observed. The desmosomes in the rat trachea seemed to be shorter than those found in the human tissue. Hemidesmosome attachments were observed between basal cells and the basal lamina. The columnar cells send out basal extensions that are closely apposed to the basal lamina, but that do not have hemidesmosomes.
Human bronchial biopsies

TEM of human respiratory epithelium showed several layers of basal cells. Tissue from patients with atopic asthma showed, in contrast to control tissue, damage ranging from minor changes up to shedding of the epithelium. In both the controls and the asthmatics, desmosomes were present between columnar cells, between basal cells, and between columnar and basal cells. Hemidesmosomes were observed attaching the basal cells to the basal lamina in both groups. As in the rat, no hemidesmosomes were observed attaching the columnar cells to the basal lamina, neither in the controls nor in the asthmatics. In the asthma patients the basal lamina was curved and thickened, with an irregular thickness. In asthma patients, widening of the interstitial space between the basal cells was observed. Goblet cell hyperplasia was a common finding in asthmatics. Intercellular spaces adjoining the basal lamina were larger in the human airway epithelium compared to the rat. The only significant difference between controls and asthmatics was a decrease in contact area between columnar cells and basal lamina.

Paper III: Ultrastructural investigation of cilia in PCD and SCD patients

After examining all the 35 samples blindly, the patients could then be divided into 2 groups: 16 PCD patients and 19 SCD patients, using as criterion the number of outer dynein arms: if this on average was less than 5, the patient was judged having PCD, if it was equal to or larger than 5, the patient was judged to have SCD. No significant difference in distribution between cell types (ciliated cells, cells with a few cilia, non-ciliated, and goblet cells) between PCD patients and SCD patients could be observed.

TEM of normal cilia showed the conventional “9+2” structure of the cilia, dynein arms are present. In the cilia of a PCD patient the dynein arms were missing. The average number of inner respectively outer dynein arms per cilia for the PCD patients was 1.4 respectively 1.5, for the SCD patients 5.9 respectively 8.1, and the controls had 5.2 respectively 7.9 inner respectively outer dynein arms. PCD patients had significantly (p < 0.0001) fewer inner and outer dynein arm-like structures compared to SCD patients and healthy controls. There was no significant difference between SCD patients and healthy controls. Calculating the means of the number of inner / outer dynein arms per cilia after consecutive measurements on 1 to 30 cilia for a PCD patient and an SCD patient, concluded that the measurement of 10-15 cilia is enough to make a good estimate of the number of outer dynein arms and a diagnosis.

Compared to PCD patients, SCD patients have more cilia with structural abnormalities such as compound cilia, missing peripheral or central micro-
tubules, or missing membranes. Supernumerary microtubules were more common in PCD patients. Membrane blebs and microtubule dislocation were similar in both PCD and SCD patients.

The mean index of COR in the PCD group was $43.6 \pm 12.9^\circ$ (range 17.6 to 70.9$^\circ$) and in the SCD group it was $21.8 \pm 11.3^\circ$ (range 10.9 to 43.6$^\circ$) ($p<0.001$). Even though the distribution of orientation angles was significantly more regular in SCD patients, because of the overlapping values it is difficult to use this criterion to distinguish between PCD and SCD patients. The same conclusion was reached when the PCD and SCD groups were subdivided into paediatric and adult subgroups. Both for the paediatric and the adult patients, there was a significant difference in COR depending on diagnosis, but also a clear overlap. There was no significant difference between paediatric patients and adult patients with the same diagnosis.

Paper IV: Effects of corticosteroids and leukotrien receptor antagonists on airway epithelial cells

Apoptotic and necrotic cells

Exposure of 16HBE cells for 24 or 48 h to dexamethasone resulted in a concentration-dependent apoptosis, reaching a maximal effect at a dexamethasone concentration of 0.5 $\mu$M. Dexamethasone caused a minor, but significant necrosis of 16HBE cells after 24 h of incubation, but this effect was not found after 48 h of incubation. Beclomethasone did not cause significant apoptosis or necrosis of 16HBE cells during a 16-h incubation period, whereas budenoside only caused apoptosis or necrosis at the lowest concentrations used, 0.1 and 0.5 $\mu$M. Montelukast caused both apoptosis and necrosis of 16HBE cells, but to a lower extent than dexamethasone. Studies on time-dependence of apoptosis and necrosis showed that the effect of dexamethasone was relatively larger at shorter incubation times (up to 16 h), whereas the effect of budesonide and beclomethasone progressed at least up to 24 h. The effect of montelukast on apoptosis or necrosis in time was somewhat reduced compared to that of the corticosteroids. The apoptotic effect of corticosteroids on 16HBE-cells could not be inhibited by the pancaspase inhibitor Z-VAD-fmk.

It was only possible to test low concentrations of corticosteroids and montelukast on Calu-3 airway epithelial cells, since the substances had to be dissolved in ethanol and Calu-3 cells were more sensitive to ethanol than 16HBE cells. The effects of corticosteroids and montelukast on apoptosis and necrosis of Calu-3 cells were much less than on 16HBE cells and these effects were not inhibited by Z-VAD-fmk.
Effect of corticosteroids and cytokines on desmosomes in airway epithelial cells

TEM showed that treatment of 16HBE cells with TNF-α or interferon-γ reduced the fraction of cell membrane length that was made up of desmosomes. This reduction was counteracted by treatment with corticosteroids.

Effect of corticosteroids and montelukast on ICAM-1 expression

Treatment of 16HBE cells with corticosteroids or montelukast reduced the expression of ICAM-1, but in contrast, in HUVEC this treatment resulted in an increase of ICAM-1 expression.
DISCUSSION

Studies on human tissues in situ (Papers I, II, III)

Bronchial and nasal brush biopsies in the diagnosis of airway diseases (asthma and PCD) and diseases with respiratory symptoms (Gaucher disease)

Lysosomal changes in the respiratory epithelium (Paper I)

Respiratory system involvement in type 2 Gaucher disease is common (but rare in other types of Gaucher disease), and is generally assumed to be linked to the presence of the so-called Gaucher cells in the lung. Gaucher cells are cells of the reticuloendothelial system containing abundant lysosomal bodies. These cells are found in the interlobular septa, around pulmonary vessels and bronchioles (Amir & Ron, 1999) and also in bronchoalveolar lavage (Carson et al., 1994). It has been suggested that the Gaucher cells can fill the alveolar spaces and/or the inter- and intralobular septa, leading to air space and/or interstitial disease (Santamaria et al., 1998).

Even in lysosomal storage diseases with manifestations in the central nervous system, the finding of cells with abundant lysosomal bodies outside the brain, often in peripheral nerve cells, is common (Ceuterick-de Groote & Martin, 1998). A considerable, disease-specific heterogeneity exists in the sites in which these cells can be found and in the ultrastructure of the lysosomal bodies. Skin biopsies have proven to be a good tool in the diagnosis of lysosomal storage diseases (Prasad et al., 1996). Biopsies from the conducting airways are not normally taken from patients with suspected lysosomal storage diseases, and no data are available from such tissues. However, in a mouse model of type B Niemann-Pick disease, abnormal lysosomal lipid storage was noted in the ciliated airway epithelial cells (Dhami et al., 2001).
The lack of conspicuously abnormal lysosomes in the nasal biopsy may be due to a more rapid turnover of nasal epithelial cells, preventing accumulation of membrane material in the lysosomes. The ultrastructural data point to considerable damage to the epithelium of the lower airways. It cannot be excluded that the ciliary dyskinesia resulting from this damage, rather than the usually presumed presence of Gaucher cells in the lung interstitium, can explain the respiratory symptoms in this particular patient and patients with type 2 Gaucher disease in general.

**Human vs. rat airway epithelia (Paper II)**

The structure of the respiratory epithelium in a number of animal species was investigated quantitatively by Evans et al. and Plopper et al., but no data on human epithelium were included (Evans et al., 1989, 1990; Plopper et al., 1980). The measurements on rat respiratory epithelium in the present study were primarily carried out to see whether our methods of determining quantitative parameters agreed with those of Evans et al. (1989). This is indeed the case. In the rat, for the columnar cell height values ranging from 11.5-16 µm were found by Evans et al. (1989), and an average value of 14 µm by Evans et al. (1990). The number of basal cells per 100 µm basal lamina was between 5 and 7 in the rat (Evans et al., 1989). This agrees well with our data. When different species were compared, a nearly linear relationship between the height of the columnar cells and the number of basal cells per 100 µm could be shown, as well as a positive relationship between the number of basal cells per 100 µm and the contact area between basal cells and basal lamina, and between basal cells and columnar cells, respectively (Evans et al., 1989). We find that compared to the rat, the height of the columnar cells in the human respiratory epithelium is significantly increased. There are also more basal cells in the human epithelium, and a larger contact area between basal cells and the basal lamina, as well as between basal cells and columnar cells. As a logical consequence, the contact area between columnar cells and basal lamina is decreased in the human, compared to the rat. The values for the human respiratory epithelium are close to those for the monkey (columnar cell height 18-25 µm and 9-12 basal cells/100 µm basal lamina) given by Evans et al. (1989).

**Cell attachments (Paper II)**

In agreement with previous studies we found that the airway epithelial columnar cells form desmosome attachments with adjacent cells (columnar and basal) but do not form hemidesmosome attachments with the basal lamina (Christensen et al., 1987; Dalén, 1983). This is an important point because it indicates that there are no junctional attachments between columnar cells and the basal lamina. Columnar cells attach directly to the basal lamina by means of slender processes, and indirectly by forming desmosome attachments with the basal cells. However, in shorter epithelia such as that of the
rat, there are fewer basal cells and there is more contact between columnar cells and basal lamina. In the human epithelium, the desmosomal contact between basal cells and columnar cells is the most important mechanism for preserving the integrity of the respiratory epithelium, and an important role of the basal cells is to keep the columnar cells attached to the basal lamina. Whether the basal cells also serve as stem cells for the airway epithelium, or whether the stem cells of the respiratory epithelium are to be found in the glands, is still a matter of controversy (Engelhardt et al., 1995; Borthwick et al., 2001; Hong et al., 2004a,b). The basal cells observed in the material of the present study are of the “larger airway” type described by Nakajima et al. (1998), with a prominent cytoskeleton and relatively many hemidesmosomes.

The present study found that the percentage of the columnar cell attachment surface in direct contact with the basal lamina was significantly reduced in asthmatics. In a previous study, we have shown that desmosomes are smaller in asthma patients as compared to the controls, and that half-desmosomes (i.e., desmosomes with a plaque only in one cell) could be found frequently in asthma patients (Shahana et al., 2005). It has to be pointed out that in the present study, measurements were carried out in grossly intact regions of the epithelium; in agreement with many other studies we observed considerable detachment of the bronchial epithelium in asthma, leaving only a layer of basal or reserve cells on the basal lamina (Laitinen & Laitinen, 1994a). It is therefore of interest that already in early stages of damage, the contact between columnar cells and basal lamina is decreased. This, in combination with the decrease in size of the desmosomes that we observed in another study (Shahana et al., 2005) could be an important factor in the development of epithelial shedding in asthma. The data support the notion of Knight and Holgate (2003) that there is an intrinsic weakness in the airway epithelium of asthmatics.

**Basal lamina (Paper II)**

Another difference that has been described earlier is the thickened and curved structure of the basal lamina in asthma patients (Laitinen & Laitinen, 1994a); this explains why the length of the basal lamina in the same field of view in asthma patients was 99 µm compared to 81 µm for the controls.

In conclusion, this study clearly demonstrates that basal cells in the pseudo-stratified epithelium of the human bronchi are involved in indirect attachment of columnar cells to the basal lamina. Presumably, this is in order to maintain or increase the tensile strength of the epithelial-basal lamina attachment in taller epithelia. The taller the epithelium, the more important is the indirect attachment of the columnar cells to the basal lamina (columnar
cell attachment to basal cells). A statistically significant difference was found between asthma patients and controls in the percentage of basal lamina in direct contact with columnar cell surface. The decrease of this contact, found in asthma patients, may be a contributing factor in epithelial shedding.

The study also shows a significant difference in the extent of attachment of columnar cells to the basal lamina in rats versus humans. The implication of this would be that the human airway is more prone to epithelial shedding, and that the rat therefore is not an optimal model for studies of airway inflammation, if epithelial shedding is an important aspect of these investigations (Dorscheid et al., 2003; Dosanjh et al., 2001; Sutton et al., 2000; Hussain et al., 2004; Sarpong et al., 2003).

**Diagnostic criteria between PCD and SCD (Paper III)**

The diagnosis of PCD must be based on the patient’s clinical history. It was noted from the review of the patients’ charts, that most of them had signs and symptoms suggestive of PCD, although more than half of the samples examined proved to be SCD cases. PCD should be suspected in all patients with chronic respiratory symptoms, bronchiectasis, situs inversus, unexplained neonatal respiratory distress, or a family history of PCD (Coren et al., 2002). Before the performance of specific tests to establish the diagnosis of PCD, the most common causes of respiratory disease should be ruled out. The diagnosis may be based on an abnormal saccharine test, but its confirmation depends at present on abnormal structure of cilia or abnormal ciliary function (Jorissen et al., 2000a), although nasal NO levels (Kharitonov, 2004) and genetic analysis may provide valuable information. Diagnostic delay leading to inadequate therapy may result in poorer outcomes for patients with PCD (Coren et al., 2002). It is therefore of great importance to differentiate between PCD and SCD patients.

Since we found no significant difference in the distribution of different cell types between PCD and SCD patients, this parameter is not important in the diagnosis of PCD. On the other hand, it is essential to evaluate the sample regarding cell type, to confirm that it is representative and contains a reasonable number of ciliated cells, in order to establish a correct diagnosis. A report of aspecific ciliary damage involving less than 5% of the epithelial cells would suggest an acquired disorder. However, the loss of differentiated epithelial cells and the ultrastructural defects of the ciliary system are much larger in PCD than in SCD patients (Grella et al., 2001).

In healthy tissue, it is possible to identify between 7 and 9 outer dynein arms and 4 and 7 inner dynein arms per ciliary section (Jorissen et al., 2000b,c). In our study we found that the number of outer dynein arms in healthy controls was on average 8 and the number of inner dynein arms was
5 per ciliary section, which is in agreement with the findings of Jorissen and co-workers (Jorissen et al., 2000b,c). In PCD patients inner, outer, or both dynein arms may be defective, and the absence of dynein arms may be partial or complete (Escudier et al., 2002). It is not necessary to measure the number of dynein arms in a large number of cilia to make a diagnosis. According to this finding we measured the number of dynein arms in 10 ciliary cross-sections per sample.

Other abnormalities have been reported in PCD cases, but most of these abnormalities can also be found in cases of SCD (Jorissen et al., 2000d). These other defects include absence of radial spokes, ciliary disorientation, and ciliary transposition (Noone et al., 2004). The ultrastructural defects of the ciliary axoneme that we assessed in this study are the ones most often observed in PCD and SCD: absence of inner and/or outer dynein arms, missing peripheral or central microtubules, extra microtubules, dislocated microtubules, compound cilia, and membrane evaginations. Missing nexin links have been described as possible congenital ciliary defects, although definitive confirmation is still needed (Armengot Carceller et al., 2005). In agreement with others, we found that peripheral microtubule abnormalities are more common in SCD than PCD patients. SCD patients have more cilia with missing central or peripheral microtubules, missing membranes, or have compound cilia. However, contrary to others we found that membrane evaginations were similar in both PCD and SCD patients (Afzelius et al., 1983; Carson et al., 1985; Pizzi et al., 2003). According to Jorissen and co-workers (Jorissen et al., 2000d), membrane blebs are the most frequently encountered SCD abnormality.

The direction of the ciliary beat is given by the orientation of the central pair of microtubules. In normally beating cilia, the orientation of the central pair should be similar within 20 degrees (Jorissen & Willems, 2004). Even though the distribution of orientation angles was slightly more regular in SCD patients in the present study, this parameter could not be used to distinguish between PCD and SCD. It could be suspected that the ciliary orientation is compromised by the brush biopsy procedure (Jorissen & Willems, 2004), but this was not found to be the case by Rautiainen and co-workers (Rautiainen et al., 1990). COR was not found to be dependent on the age of the patients with the same diagnosis, which is in agreement with findings by Biggart and co-workers (Biggart et al., 2001).

This study revealed that quantitative and qualitative ultrastructural assessment of respiratory epithelial cilia plays an important role in the differentiation between primary, secondary, and borderline ciliary abnormalities. This is in agreement with Pizzi and co-workers (Pizzi et al., 2003). Early
diagnosis of PCD with appropriate clinical follow-up and treatment is important to prevent irreversible lung tissue damage.

In summary we conclude that, patients with SCD can be distinguished from patients with PCD by checking the number of dynein arms (or dynein-arm like structures) in “true” cross sections of cilia. It is not necessary to examine many cilia if the cross-sections are at a right angle to the ciliary axis. SCD patients appear to have more often compound cilia than PCD patients (it should be noted that compound cilia can be caused by the use of nasal decongestant) (Roomans et al., 1983). The cilia in SCD patients also tend to have missing membranes, and/or missing peripheral or central microtubules, but fewer supernumerary microtubules than PCD patients.

Experimental studies (Paper IV)

Effects of corticosteroids, montelukast, and cytokines on bronchial epithelial cells:

**Apoptosis and necrosis**

Our results indicate that corticosteroids cause apoptosis and necrosis of airway epithelial cells and reduce the expression of desmosomes and ICAM-1. Many studies have been carried out to determine the effect of corticosteroids on airway epithelial cells, but with variable results. Studies by various groups (Dorscheid et al., 2001; White & Dorscheid, 2002; Tse et al., 2003) showed that corticosteroids promoted apoptosis in airway epithelial cell lines. In vitro studies on nasal polyp tissue showed that corticosteroid treatment caused an increase in apoptosis (Saunders et al., 1999). In vivo studies on experimental animals and patients showed an increased loss of epithelial cells and an increased presence of epithelial cells in the bronchiolar lavage fluid (Dorscheid et al., 2003; Pavlovic et al., 1998; Benayoun et al., 2001, 2003), indicating an increase in apoptosis and/or necrosis. On the other hand, several studies (Laitinen et al., 1991, 1992; Vignola et al., 2001) did not find an increase in the number of apoptotic cells in asthmatics treated with corticosteroids, and it was found that treatment with corticosteroids resulted in a thicker airway epithelium (Baroody et al., 2001), which would indicate that corticosteroids would protect against apoptosis and necrosis. We found a maximal effect at a corticosteroid concentration of about 0.5 µM, which agrees with the fact that corticosteroid receptors are saturated at a concentration of 0.3 µM (Hamelmann & Schleimer, 2003). We also found that the corticosteroid-induced apoptosis of airway epithelial cells could not be in-
hibited by Z-VAD-fmk, which would imply that the apoptosis is independent of caspase-3, -4 or -7, but according to Dorscheid and coworkers, this apoptosis is dependent on caspase-9 (Dorscheid et al., 2001; White & Dorscheid, 2002).

There is general agreement on the fact that corticosteroids promote apoptosis of inflammatory cells (Drulilhe et al., 2003), as do leukotrien receptor antagonists such as montelukast (Spinozzi et al., 2004; Abadoglu et al., 2005). On the other hand, the effect of leukotrien receptor antagonists on apoptosis and necrosis of airway epithelial cells has to our knowledge not been studied previously. We found that the leukotrien receptor antagonist promoted apoptosis and necrosis in these cells, but to a lesser extent than corticosteroids.

Desmosomes
Proinflammatory cytokines such as TNF-α and IFN-γ play a central role in the initiation of epithelial damage in asthma (Broide et al., 1992). Kampf et al. (Kampf et al., 1999) showed that these cytokines caused a reduced expression of desmosomes, and Carayol et al. (Carayol et al., 2002a,b) showed that TNF-α caused a reduced expression of other adhesion molecules such as E-cadherin, β-catenin and plakoglobin, in airway epithelial cells. Our data confirm this effect of TNF-α and IFN-γ, and show that this effect is inhibited by corticosteroids. This finding is in agreement with other studies (Carayol et al., 2002b). We attempted to determine by immunocytochemistry whether a particular protein forming part of the desmosomes was specifically affected by proinflammatory cytokines and/or corticosteroids, but the results of these experiments were not conclusive.

ICAM expression
We found that both corticosteroids and leukotrien receptor antagonists reduced the expression of ICAM-1 on airway epithelial cells. This agrees with studies that found that corticosteroids reduced the expression of ICAM-1 in bronchial epithelial cells (Van de Stolpe et al., 1993), lung cancer cells (Paolari et al., 1997), but not in renal epithelial cells (Wuthrich & Sekar, 1993) and nasal polyps (Doner et al., 2004). An advantage of reduced ICAM-1 levels would be that ICAM-1 functions as a receptor for rhinovirus, and reduced levels would thus provide a protective effect against rhinovirus infection which often causes complications for asthma patients (Papi et al., 2000; Papadopoulos et al., 2004). The effect of montelukast on ICAM-1 has not been studied directly previously, but it has been shown that montelukast inhibits cysteinyi leukotrien-induced eosinophil motility (Fregonese et al., 2002) and reduces levels of soluble ICAM-1 in plasma in asthmatics (Stelmach et al., 2002), which indicates that montelukast can affect the expression of ICAM-1. Also the effect of corticosteroids on ICAM-1 expression in HUVEC has been studied previously, and in general an inhibitory effect of
corticosteroids on the increased expression of ICAM-1 induced by endotoxin or proinflammatory cytokines was found (Cronstein et al., 1992; Aziz & Wakefield, 1996; Ihm et al., 1996; Wheller & Peretti, 1997), which does not match our findings of increased ICAM-1 expression in HUVEC exposed to corticosteroids.

In conclusion, both corticosteroids and leukotrien receptor antagonists increase apoptosis and necrosis in cultured airway epithelial cells, but leukotrien receptor antagonists have smaller effects than corticosteroids, which may be an advantage in clinical practice. Desmosomes between airway epithelial cells are weakened in asthmatics, probably due to the presence of pro-inflammatory cytokines, and corticosteroids restore these desmosomes. Finally, both corticosteroids and leukotrien receptor antagonists reduce the expression of ICAM-1 on airway epithelial cells but increase the expression of this adhesion molecule on endothelial cells.
CONCLUSIONS

In this thesis different airway diseases have been studied in depth with the help of electron microscopy to improve our understanding of the diseases, which is also necessary for an optimal clinical treatment.

- A tracheal biopsy from a paediatric patient with respiratory symptoms revealed lysosomal changes, suggesting the diagnosis of a metabolic disorder.

- The percentage of basal lamina in direct contact with the columnar cell surface is decreased in asthmatics compared to healthy persons.

- The structure of the airway epithelium of rats differed significantly from that in humans. The structure of the airway epithelium of humans is close to published data for the monkey. The use of rats as an animal model for the study of diseases affecting the airway epithelium is therefore not highly reliable.

- The number of dynein-arm-like structures can be used to distinguish between patients with PCD and patients with SCD. It is not necessary to examine many cilia if the cross-sections are at a right angle to the ciliary axis.

- Both corticosteroids and montelukast cause apoptosis and necrosis of airway epithelial cells, but montelukast did this to a lesser extent.

- Corticosteroids inhibit the effect of pro-inflammatory cytokines on desmosomal contacts between airway epithelial cells.

- Both corticosteroids and montelukast reduced the expression of ICAM-1, but in cultured HUVEC cells treatment with corticosteroids or montelukast caused an increase in ICAM-1 expression.
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