Experimental Lung Research, 27:401–415, 2001

0190-2148/01 $12.00 + .00

MINI-REVIEW

CELLULAR AND MOLECULAR CHARACTERISTICS OF BASAL CELLS IN AIRWAY EPITHELIUM

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☐ *Basal cells exist as a separate layer of cells covering most of the airway basal lamina. In this central position, they can interact with columnar epithelium, neurons, basement membrane, and the underlying mesenchymal cells. In addition, they interact with inflammatory cells, lymphocytes and dendritic cells. These interactions take place in the lateral intercellular space between basal cells. In this central position basal cells become a very important part of the epithelial-mesenchymal trophic unit of larger airways. In this review it is shown that basal cells may function as progenitor cells of airway epithelium and have a role in attachment of columnar epithelium with the basement membrane. They also have the potential to function in regulation of neurogenic inflammation, the inflammatory response, transepithelial water movement, oxidant defense of the tissue and formation of the lateral intercellular space. Other characteristics of basal cells were not clearly associated with a particular function. The functions for basal cells listed attempt to explain the presence of recently identified molecules in basal cells of airway epithelium. It should be pointed out that specific studies have not been carried out which test the relationship between the molecular functions we describe in this review and the basal cell in airway epithelium.*

Keywords *airway epithelium, lateral intercellular space, inflammation, progenitor cell, epithelial-mesenchymal, epithelial attachment*

BACKGROUND

In a previous review, the origin, distribution, and morphology of airway basal cells in a number of different animal species and at different airway levels were summarized [1]. Basal cells were characterized by their basal position in the columnar epithelium, the presence of hemidesmosomes,

Received 22 August 2000; accepted 11 September 2000.

This work was supported by NIH grants ES00628, ES04311, ES06700, ES05707, UCD Health System Research Program, California Tobacco Related Diseases Research Program Grant 6KT-0306, American Lung Association, and EPA Grant R827442-01-0.

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cytokeratins 5 and 14, and reaction with the lectin *Griffonia simplicifolia* isolectin B_4. It was shown that basal cells were derived from undifferentiated columnar epithelium in a number of animal species, including humans. The distribution of basal cells in airway epithelium was different at various airway levels and in different animal species. Airways larger in diameter had a taller epithelium and more basal cells than airways smaller in diameter with a shorter epithelium. For example, the largest numbers of basal cells were found in the trachea. As the airway decreased in diameter, the number of basal cells also decreased and none were present in the terminal bronchioles of any animal species studied. The distribution of basal cells at a particular airway level also varied according to the species. For example, in the hamster trachea there were 39 basal cells/mm, in the monkey trachea 102 basal cells/mm, and in the sheep trachea 197 basal cells/mm. Despite these differences, there was a unifying feature of basal cell distribution in all of the animal species studied, that is, the number of basal cells present was related to the height of the columnar epithelium rather than animal species, airway level, or the number of columnar cells present. From these studies it was shown that a major function of airway basal cells is to attach the columnar epithelium to the basement membrane. It was also concluded that basal cells act as progenitor cells for airway epithelium under certain conditions. Since the previous review, new evidence has accumulated indicating that basal cells may also play an important role in regulating physiologic and inflammatory responses of the airway.

The potential importance of basal cells as regulatory cells is highlighted by several anatomical characteristics of airway epithelium. As airways increase in diameter, basal cells increase in number and displace columnar cells on the basal lamina [2–4]. In human airways, 90% of the basal lamina is covered by basal cells in bronchi that are larger than 1 to 3 mm in diameter [2]. Although columnar cells make contact with the basal lamina in the larger airways, functionally the tissue is a stratified epithelium, with a layer of basal cells attached to the basal lamina and a layer of columnar epithelium attached to the basal cells [1]. Thus basal cells are a separate layer of cells, in a central position, that can interact with columnar epithelium, neurons, basement membrane, and the underlying mesenchymal cells. In addition, they can interact with inflammatory cells, lymphocytes, and dendritic cells in the epithelium. These interactions take place in the lateral intercellular space between basal cells (Figure 1). In this central position basal cells become a very important and integral part of the epithelial-mesenchymal trophic unit of larger airways [5].

Since the last review [1], a number of molecules have been associated with basal cells (Table 1). Most of these studies described the distribution of the molecules in the whole airway and not with respect to the function of basal cells. Some of these molecules suggest the basal cell is involved

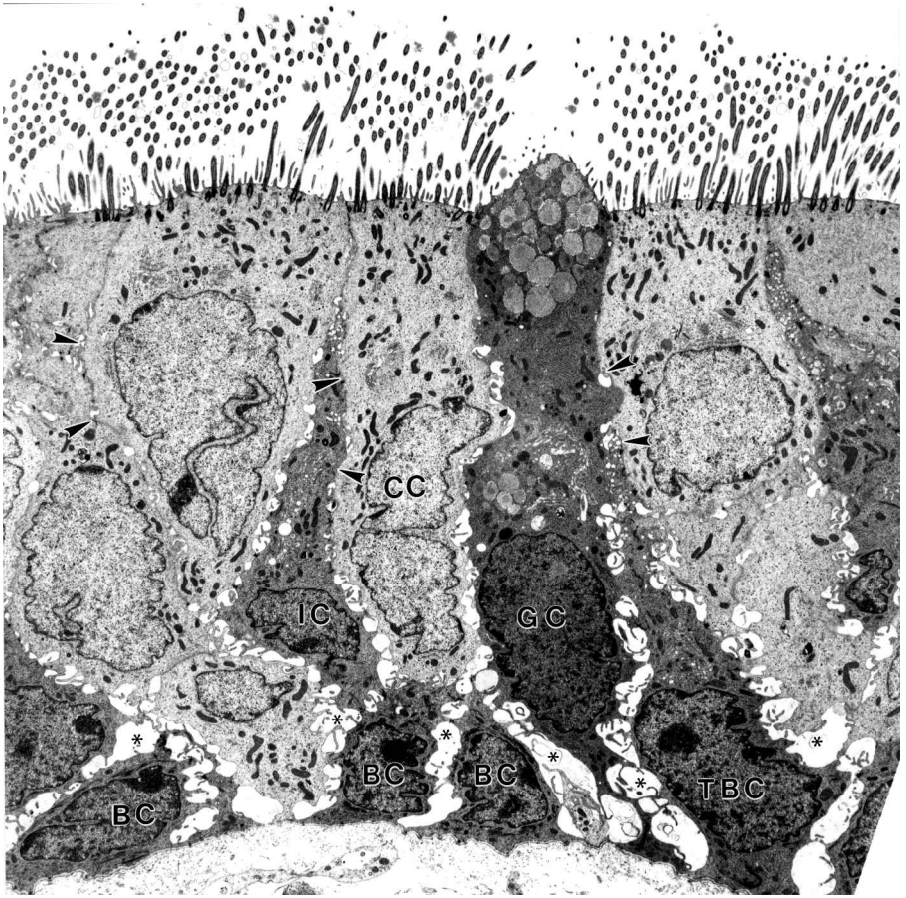

FIGURE 1 Electron micrograph of tracheal epithelium from a young Rhesus monkey, demonstrating basal cells (BC), tall basal cells (TBC), ciliated cells (CC), goblet cells (GC), indeterminate cells (IC), and the lateral intercellular space (LIS). LIS is the open area between cells (*asterisks*). The LIS is reduced or absent between ciliated cells and ciliated cells next to secretory cells (*arrowheads*).

with specific functions such as (a) regulation of neurogenic inflammation; (b) the response of inflammatory cells; (c) transepithelial water movement; (d) oxidant defense of the tissue; and (e) formation of the lateral intercellular space. Other molecular characteristics of the basal cell are not clearly related to specific functions. In this review we will discuss new information concerning basal cells as progenitor cells, junctional adhesion, and possible functions of the basal cell with respect to the molecules associated with them.

FUNCTIONS OF BASAL CELLS

Progenitor Cells

The basal cell has the capacity to be the progenitor of columnar airway epithelium under certain conditions. This has been clearly demonstrated in

TABLE 1 Cellular and Molecular Characteristics of Basal Cells

Cell surface characteristics	Reference
Alkaline phosphatase	19
Aquaporin 3 transmembrane water channels	49
β-Adrenergic receptors	73
CD44 transmembrane glycoproteins	59
Epidermal growth factor receptor	61, 64
Fas receptors and ligand	47, 48
ICAM-1	42
IgE receptor	43
Integrins .α6β4/	76
Lectins	77, 78
LEEP-CAM	44
MRP transmembrane transporters	54
Neutral endopeptidase	39
4-1BB receptor	45
Intracellular characteristics	**Reference**
Adrenomedullin receptor (mRNA)	55, 56
Autotaxin (mRNA)	74
Annexin II	70
Bcl-2 protein	48
Extracellular SOD (mRNA)	52
Leukemia inhibitory factor	40

studies where denuded airways were repopulated with enriched populations of basal cells [6–9], in biphasic organotypic cultures [10], and in vivo following loss of the columnar epithelium [11]. Under these conditions basal cells ''dedifferentiate'' into a highly proliferative cell phenotype from which a mucociliary epithelium ''redifferentiated.'' Basal cells have also been shown to be the progenitors of primary rat tracheal [12, 13] and human bronchiolar epithelial cell cultures [14].

Recently in human airway epithelium, proliferation of 2 populations of basal cells (basal and parabasal cells) were described by Boers and colleagues [15]. They used the antikeratin antibody 34BE12, which is specific for cytokeratins 1/10 and 5/14, to identify the basal cells. Basal cells had their nuclei next to the basement membrane; parabasal cells were columnar and tall, similar to indeterminate cells, and their nuclei were above the layer of basal cell nuclei. Basal cells made up 31% of the cell population in large airways and parabasal cells 7%. Other investigators have also described 2 populations of basal cells [16–20], supporting the concept of a second tall basal cell population. The overall proliferative fraction of basal cells was 0.87%. When separated from the rest of the epithelium, basal cells had a proliferative fraction of 1.69% and parabasal cells 5.25%. However, because of the low percentage of parabasal cells in the epithelium, they only made up 0.27% in the proliferative compartment. The authors used an antibody to the proliferation-associated protein Ki-67 to determine the proliferative fraction of cells in the airways. Ki-67 is present in all phases

of the cell cycle except G_0 [21]. The proliferative fraction obtained with Ki-67 staining contains cycling cells plus those arrested in various phases of the cell cycle. The proliferative fraction obtained by this technique is several times larger than one obtained with 3H-TdR or BrdU labeling techniques. However the higher proliferative fraction in parabasal cells suggests they may be the indeterminate columnar cells described by others. The indeterminate cell population is active in reparative proliferation following injury when compared with basal cells [22]. If the parabasal cells prove to be the indeterminate cells, it would support the concept of an in vivo basal cell type as progenitors of the columnar epithelium. However at this time there is not enough information to determine if parabasal cells are the same as indeterminate cells.

In the normal adult airway epithelium, the rate of basal cell proliferation is low [1, 15, 23]. Some of this proliferation may be associated with cell turnover of the columnar epithelium. Proliferation may also be for replacement of dying basal cells. When basal cells are lost due to injury, proliferation of surviving basal cells occurs and they are replaced, suggesting that the low rate of proliferation in adults may be for this purpose [24]. However senescence of the basal cell population has not been studied and the normal death rate of basal cells is not known.

During growth of the airway the basal cell has a high rate of proliferation [1, 25]. The purpose of such proliferation in the growing rat trachea is for the formation of new basal cells [26, 27]. For example in the growing rat, the trachea increases in circumference from 2.5 to 7.5 mm between 3 and 90 days of age. During this time the total basal cell population increases 9 fold in size. Proliferative indices in the literature predict an initial doubling time of 14 days for the basal cell population, which gets progressively longer as the animal ages [1, 23]. The basal cell population doubling times are in agreement with the large increase in the total number of basal cells seen in the growing airway. The increase in basal cells per millimeter of basal lamina is related to their role in attaching columnar epithelium to the basal lamina [26].

Junctional Adhesion

The structural role of basal cells in the airways is for attachment of columnar epithelium to the basal lamina. Epithelial cells are attached to the basal lamina by hemidesmosomes and cell adhesion molecules. Anchoring junctions (desmosomes and hemidesmosomes) join the cytoskeletons of adjacent cells together and attach them to the basal lamina. This arrangement of junctional adhesion provides mechanical stability to a group of cells or tissue. In airway epithelium, basal cells are the only cell type that form hemidesmosome junctions with the basal lamina [1]. Columnar cells are

attached to the basal lamina via desmosome attachment with basal cells. In smaller airways, where basal cells are not present, columnar cells are attached to the basal lamina with cell adhesion molecules. Defining the mechanism of columnar cell attachment to the basal lamina is critical to the understanding of asthma and other disease conditions associated with sloughing of epithelium. In conditions where columnar cells are sloughed from the epithelium of larger airways, the basal cells remain attached to the basal lamina [28–30]. Desmosomal attachment between the columnar epithelium and basal cells represents a plane of cleavage between the 2 cell populations. Failure of the desmosomal attachment between columnar and basal cells is thought to be responsible for sloughing of the columnar epithelium in asthmatics [28, 31, 32]. The mechanism of desmosomal failure between airway cells is not known. Erjefalt and colleagues [33, 34] speculated that it is not a failure per se but rather a specific protective function of basal cells. They found that within 20 minutes after the columnar epithelium had been removed, basal cells had flattened out and formed a protective barrier. They considered this an important protective function of the basal cell along with the ability to quickly release desmosomal attachments to damaged columnar epithelial cells. It may also be speculated that stimulation of the epithelial neural system causes edema of the lateral intercellular space with a subsequent local sloughing of the epithelium [35]. In a similar manner an influx of inflammatory cells could overwhelm the lateral intercellular space and cause local sloughing of the epithelium [36, 37]. However, these scenarios are speculation and the reasons for, or mechanism of, epithelial sloughing is not known at this time.

Neurogenic Inflammation

In response to various inhaled foreign materials, axons in the airway epithelium release neuropeptides into the lateral intercellular space, initiating the process of neurogenic inflammation (increased vascular permeability, neutrophil adhesion, vasodilatation, gland secretion; ion transport; smooth muscle contraction; increased cholinergic transmission; cough) [38–39]. Basal cells contain the protein leukemic inhibitory factor (LIF). LIF is thought to function in neurogenic inflammation by stimulating release of neuropeptides (tachykinins) from axons and formation of neurokinin receptors [40]. Neutral endopeptidase (NEP) is a cell surface enzyme also associated with the process of neurogenic inflammation. NEP cleaves neuropeptides in the lateral intercellular space. Cleavage of neuropeptides by NEP modulates the neurogenic inflammatory responses in the airways. NEP is expressed mainly on the surface of basal cells and not columnar cells in normal rat airways [39]. In asthmatic human subjects, NEP is also expressed intracellularly in columnar cells [41]. Because basal cells contain

the protein LIF and express NEP on their surface, it suggests that one of their regulatory functions is modulation of the neurogenic inflammatory response of the airways.

Inflammation

Basal cells participate in the inflammatory response by upregulating expression of receptors for migratory inflammatory cells and lymphocytes. Human basal cells upregulate intercellular adhesion molecule-1 (ICAM-1) [42]. Indeterminate cells also express ICAM-1 but ciliated and goblet cells do not. Zhu and colleagues [42] conclude that basal and indeterminate cells are likely to play key roles in leukocyte retention via upregulation of ICAM-1. Human basal cells can also upregulate expression of immunoglobulin E (IgE) receptors, indicating they may be involved with allergic responses of the airway [43]. An unusual cell adhesion molecule, lymphocyte endothelial-epithelial cell adhesion molecule (LEEP-CAM), is expressed in the basal cell layer of human bronchial epithelium. Lymphocyte adhesion to epithelia and endothelia is mediated by LEEP-CAM [44]. Human basal cells also express the receptor 4-1BB, but the columnar cells do not [45]. The receptor 4-1BB is a member of the tumor necrosis factor receptor superfamily, and is associated with T-cell activation [46]. These 2 studies [44, 45] indicate that basal cells are associated with lymphocyte movement and activation. Human basal and columnar cells both express the Fas receptor and its ligand FasL [47, 48]. Ligation of the Fas receptor by migratory inflammatory cells can lead to their apoptosis. The authors suggest that expression of these molecules by basal and columnar epithelium may play an important role in regulation of the inflammatory response. These studies demonstrate that basal cells may interact with inflammatory cells when the latter are moving through lateral intercellular space of airway epithelium.

Transepithelial Water Movement

Rat basal cells have an aquaporin water channel (AQP3) not found in the columnar epithelial cells. Columnar epithelial cells have the aquaporin water channel AQP4 [49]. Water transfer between cells and the matrix occurs through these water channels. The presence of AQP3 water channels in the membranes of basal cells of normal airway epithelium demonstrates a unique role for the basal cell in fluid modulation of airway surface liquids and also the lateral intercellular space. AQP3 is found in a basolateral position in the airways and in other tissues, implying movement of water between the extracellular matrix and epithelium [50]. The fact that basal cells cover most of the airway basement membrane and express AQP3 is in agreement with this concept. The cellular distribution of AQPs 3 and 4 in airway epithelium

demonstrate the presence of cell-specific pathways for transcellular water movement between the extracellular matrix and epithelium. It was recently shown that cystic fibrosis transmembrane conductance regulator protein is a regulator of AQP3 water channels in airway epithelial cells [51].

Oxidant Defense of the Tissue

Su and colleagues [52] found that basal cells and secretory cells in normal human airway epithelium express extracellular superoxide dismutase (EC-SOD) mRNA. EC-SOD is a secreted protein found in the extracellular matrix responsible for metabolizing superoxide free radicals [53]. The physiological functions are not fully defined but it is thought to be critical for the protection of extracellular matrix elements against oxidative damage. Brechot and colleagues [54] evaluated the distribution of the multidrug resistance–protein (MRP) transmembrane transporter in human bronchial epithelium of normal subjects. MRP transmembrane transporter plays a major role in cell detoxification and the defense against oxidant stress via efflux of glutathione conjugates. They found that the pattern of MRP expression differed markedly according to cell type. In basal cells it was distributed over the entire circumference of the cell whereas in ciliated cells it was restricted to the basolateral surface. They concluded that there were specific roles for MRP transmembrane transporter in basal and ciliated cells. The pattern of MRP expression indicates that the glutathione conjugates from both cell types are deposited in the lateral intercellular space. Expression of EC-SOD and MRP transmembrane transporter by basal cells in normal subjects suggests basal cells participate in the defense of the tissue against oxidative stress.

Lateral Intercellular Space

The lateral intercellular space is a distinct space between cells important in the process of transepithelial water movement [55]. The lateral intercellular space was first recognized as an anatomical entity in airway epithelium by Kondo and colleagues [56]. It is found between basal cells, basal and adjacent secretory cells, and between secretory cells (Figure 1). It is not generally present between adjacent ciliated cells or between ciliated cells and secretory cells. The lateral intercellular space contains nerve fibers, dendritic cells, migratory cells, and diffusable molecules. Basal and nonciliated columnar cells extend thin lateral cytoplasmic projections into the lateral intercellular space. Interactions between basal cells and nerves, dendritic cells, migratory cells, and diffusable molecules take place in the lateral intercellular space. Pirinen and colleagues [57] demonstrated that human airway lateral intercellular space contains hyaluronan. Hyaluronan is

synthesized by an enzyme complex on the plasma membrane and extruded as long chains into intercellular spaces [58]. Hyaluronan has many functions, one of which is maintaining the hydrated state of intercellular spaces. Under strong vagal stimulation [35] or hydrostatic pressure [56], the airway lateral intercellular space of rat and dog trachea, respectively, was shown to increase dramatically in volume. However, other than these studies, very little is known about the lateral intercellular space of the airway epithelium.

As mentioned above, the human airway lateral intercellular space contains hyaluronan. Hyaluronan is bound to the cell by transmembrane CD44 adhesion molecules. CD44 adhesion molecules are expressed on the surface of basal cells of normal human airway epithelium, and are not found on columnar cells. In asthmatic human subjects there are twice as many CD44 adhesion molecules on basal cells as in normal subjects [59]. It was also shown that the lateral intercellular space between basal cells is twice as large in asthmatic subjects as in normal human subjects [60]. These studies suggest that airway basal cells are involved with maintaining the lateral intercellular space through binding of hyaluronan with CD44 adhesion molecules on their surface.

OTHER CHARACTERISTICS OF BASAL CELLS

Growth Factors

Basal cells express receptors that bind growth-regulating proteins. In vivo human basal cells express epidermal growth factor receptors [61] and the proliferation of guinea pig basal cells is controlled in part by endothelin-1 [62]. Aida and colleagues [61] showed that epidermal growth factor receptor (EGFR) is located on basal and indeterminate cells, as well as on Clara cells of human airway epithelium, but is not present on goblet or ciliated cells. These cell types have been shown to be able to act as progenitor cells and expression of EGFR is in agreement with this concept. However the proliferation rate of basal cells is low in the adult airway. Epidermal growth factor (EGF) and transforming growth factor (TGF) alpha both bind to the EGFR and have many functions, ranging from induction of DNA synthesis to stimulation of differentiated functions. EGFR and its ligands are important in regulation of epithelial cell behavior [63]. Polosa and colleagues [64] suggest that EGFR on basal cells is associated with control of their functions in the airway epithelium and not specifically cell proliferation.

Adrenomedullin

Adrenomedullin receptor mRNA is abundantly expressed in basal cells of normal human airway epithelium but not in columnar cells [65].

Adrenomedullin is a multifunctional regulatory peptide whose main physiological function in the lung is vasodilation and bronchodilation [66]. It is also thought to be involved with lung development, wound repair, and tumor progression. In human airway epithelium adrenomedullin is stored in the apical regions of ciliated cells [67]. It can be secreted into the airway lumen where it may act as a antimicrobial agent. Martinez and colleagues [65] speculate that injury to the epithelium would allow adrenomedullin to bind to the basal cells and act as a mitogen. The enzyme NEP, found on the basal cell surface [68], also cleaves adrenomedullin. This suggests possible interactions of adrenomedullin with NEP and modulation of its physiological functions on the lung. Adrenomedullin can stimulate production and release of hyaluronan, a component of the lateral intercellular space [69]. This study suggests that adrenomedullin receptor may also be associated with lateral intercellular space through binding adrenomedullin and stimulating the production and release of hyaluronan.

Annexin II

Annexin II is expressed in the basal cell layer of normal bovine airway epithelium and is not associated with columnar cells [70]. Annexins are a family of calcium- and phospholipid-binding proteins that have been implicated in a number of physiological processes, including regulation of inflammation, exocytosis, and cell proliferation [71]. Annexin II has been specifically associated with exocytosis and intracellular vesicle trafficking associated with the secretory pathway [71]. Basal cells are not considered to be secretory cells; however, they do contain membrane bound vesicles [4, 24] and presumably synthesize and secret EC-SOD (52) and LIF [40]. Annexin II has also been shown to play an important role in stabilizing CD44 adhesion molecules in the cell membrane through interactions with the CD44 adhesion molecule and the actin cytoskeleton of the cell [72]. This study suggests that annexin II may also be involved with lateral intercellular space through stabilizing CD44 adhesion molecules in the basal cell plasma membrane.

Catecholamines

Kelsen and colleagues [73] showed that both basal and columnar cells in guinea pig airway epithelium have β-adrenergic receptors, which bind catecholamines. Catecholamines initiate a variety of cell responses essential to the maintenance of airway diameter and integrity of the epithelial lining and the prevention of airway inflammation. The effects of catecholamines on ciliary beating, chloride and water transport, mucus secretion, and release of smooth muscle relaxant factor in the columnar epithelium are clear but its

function in basal cells is not known. Kelsen and colleagues [73] speculate that catecholamines may effect cell proliferation or gene expression. However at this time the significance of β-adrenergic receptors on basal cells has not been determined.

Bcl-2 and Autotaxin

Druilhe and colleagues [48] showed that 20% of human basal cells, but not columnar cells, expressed the intracellular protein Bcl-2. After treatment with steroids, it could be upregulated to 40% of the cells. Bcl-2 promotes the survival of cells by inhibiting apoptic cell death. The significance of Bcl-2 being expressed only in the basal cell population is not clear. The significance of autotaxin, a autocrine motility-stimulating factor expressed mainly in basal cells of normal human bronchial epithelium, is also not clear [74].

Markers of Basal Cells

Several studies have described specific markers for airway epithelial basal cells. Hicks and colleagues [75] isolated and characterized the integrin profile of human basal cells. The isolated basal cells could be cultured for at least 7 days. Basal cells expressed integrins α 1, 2, 3, 5; v β 5; β 1, 3; and α 6 β 4. The α 6 β 4 integrin is a component of hemidesmosomes characteristic of basal cells. Atiken and colleagues [76] characterized an IgM monoclonal antibody that specifically recognizes upper airway basal cells in rabbit, rat, sheep, pig, and human. The antibody did not react with basal cells in other tissues, suggesting it could be used as a marker for airway basal cells. Inayama and colleagues [19] showed that alkaline phosphatase activity was confined to basal cells and suggested it could be used as a marker for basal cells in rabbit airway epithelium. Lectin-histochemistry was also used to identify basal cells [77, 78]. In general, basal cells are labeled by galactose or galactosamine-specific lectins. In addition to the above markers, hemidesmosomes, cytokeratins 5 and 14, the molecules found only with basal cells under normal conditions, can also be considered as markers of basal cells (CD44, annexin II, and AQP3).

SUMMARY

Although the basal cell is an integral part of pulmonary airway epithelium, it has not been studied nearly as intensively as the ciliated and nonciliated columnar cells. The information accumulating about basal cells suggests it can perform numerous functions in the airways. Basal cells exist as a separate layer of cells covering most of the basal lamina. In

this central position, they can interact with columnar epithelium, neurons, basement membrane, and the underlying mesenchymal cells. In addition, they interact with inflammatory cells, lymphocytes, and dendritic cells. These interactions take place in the lateral intercellular space between basal cells. In this central position, basal cells become a very important part of the epithelial-mesenchymal trophic unit of larger airways [5]. In this review it was shown that basal cells may function as progenitor cells of airway epithelium and in attachment of columnar epithelium with the basement membrane. They also have the potential to function in regulation of neurogenic inflammation, the inflammatory response, transepithelial water movement, oxidant defense of the tissue, and formation of the lateral intercellular space. Other characteristics of basal cells are not clearly associated with a particular function. The functions for basal cells listed above attempt to explain the presence of the recently identified molecules in basal cells of airway epithelium reviewed in this article (Table 1). Most of the studies described the distribution of these molecules in the airways and not their function in the airway. It should be pointed out that specific studies have not been carried out to test the relationship between the molecular functions we describe and the basal cells in airway epithelium.

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