

Repair mechanisms in asthma

Stephen I. Rennard, MD Omaha, Neb.

Asthma is classically defined in terms of reversible airflow obstruction. It is now recognized that histologic abnormalities, including inflammation, are a regular feature of asthma. In addition, alterations of the structural elements of the airway wall occur regularly in asthma. These alterations include a thickened basal lamina comprised of interstitial collagens as well as alterations in the mesenchymal cell population with an accumulation of myofibroblasts. It seems likely that these connective tissue alterations in the airway wall contribute to the physiologic abnormalities of asthma. While controversial, the long-term physiologic sequela of asthma may depend in large part on these changes. The biochemical and cellular basis that leads to these changes is, as yet, unknown, but recent studies suggest that a variety of cells in the airway, including inflammatory cells and the cells of the airway epithelium, may participate in regulating this response. (J Allergy Clin Immunol 1996;98:S278-86.)

Key words: Asthma, epithelial repair mechanisms

Asthma is most often defined in physiologic terms.¹ Nevertheless, alterations in the structure of the airways now are recognized as regular features of asthma.²⁻⁵ Histologic alterations in asthma include accumulations of activated inflammatory cells. These cells are thought to have fundamental roles in the pathogenesis of asthma, contributing to both acute and chronic features.⁶ Parenchymal cells of the airways also are altered, and smooth muscle hypertrophy and glandular hyperplasia are regular features.²⁻⁵ In addition, the columnar ciliated epithelial cells that line the normal airway can undergo goblet cell and squamous metaplasia. Finally, the luminal epithelial cells often rest on an altered lamina reticularis.⁷⁻¹⁰ Although sometimes termed a "thickened basement membrane," this structure is not a true basement membrane comprising laminin and type IV collagen. Rather, it consists of interstitial collagens and glycoproteins. Together with the histologic alterations, the altered structural architecture of the asthmatic airway likely contributes to the altered function of the airway in asthma. Whereas the processes that lead to these alterations are incompletely described, this review outlines current concepts of epithelial

Abbreviations used

COPD: Chronic obstructive pulmonary disease
PGE: Prostaglandin E
TGF- β : Transforming growth factor-beta

repair and provides an overview of how abnormal repair can contribute to altered function in asthma.

Repair is necessary because the airway epithelium often is subject to injury from a variety of causes. Epithelial injury, for example, can result from such diverse agents as viruses, toxins derived from bacterial or inhaled environmental pollutants, or from mediators released from activated inflammatory cells. That injury of the airway epithelium occurs in asthma is well established. In severe asthma, marked desquamation of the airway epithelium occurs.^{5, 11} Often all columnar epithelial cells may be lost, leaving a basement membrane denuded except for a partial covering of the basal cells (Fig. 1). Such injury also occurs in milder cases of asthma. Fragments of airway epithelium often can be found in the sputum of asthmatic patients.^{12, 13} When metaplastic changes also are present, these so called creola bodies sometimes can be confused with clusters of adenocarcinoma. Individual epithelial cells can be recovered by bronchoalveolar lavage, which allows their quantification in asthma. Beasley and colleagues¹⁴ have demonstrated that epithelial cell recovery in bronchoalveolar lavage fluid correlates with airway reactivity. This suggests that epithelial injury is not

From the University of Nebraska Medical Center.

Reprint requests: Stephen I. Rennard, MD, University of Nebraska Medical Center, Pulmonary and Critical Care Medicine Section, 600 South 42 Street, Omaha, Nebraska 68198-5300.

Copyright © 1996 by Mosby-Year Book, Inc.
0091-6749/96 \$5.00 + 0 1/0/76954

only a feature of asthma but also appears to be related to asthma severity.

The mechanisms that lead to epithelial injury and epithelial cell loss in asthma are not fully understood. It seems likely that several mechanisms may be involved and may vary with differing triggers. For example, viral infection clearly can injure epithelial cells and can lead to acute worsening of asthma.^{15, 16} In contrast, allergens probably activate immune cells, resulting in the release of active mediators.⁶ Such products, including those derived from eosinophils, neutrophils, and macrophages, could contribute to epithelial damage. The variable mechanisms for injuring the airway epithelium may account in part for the variable clinical course of asthma. Regardless of the mechanism, the injured epithelium could contribute directly to the acute severity of asthma (Fig. 1). Injury of the epithelium, moreover, must be followed by repair mechanisms, and the likely consequences of these repair processes lead to the altered structure of the epithelium in asthmatic patients.

In both the rat¹⁷ and the hamster,¹⁸ repair processes are initiated very rapidly after mechanical injury and follow a very similar time course. Within hours, the epithelial defect is covered by a layer of flattened squamous-like cells.¹⁸ Mitotic figures also are present within hours of injury,¹⁷ and by 24 hours, many cells in the wound are actively dividing.¹⁸ A stratified layer of undifferentiated epithelial cells several cells thick thus accumulates at the wound over a period of 2 to 3 days. Mitosis then slows and the newly accumulated epithelial cells differentiate into columnar cells. The processes that drive epithelial cell differentiation currently are not understood. Although it is controversial whether the basal cell or the secretory cell is the precursor cell or airway cell both in development and during repair, Shimizu and colleagues¹⁹ have provided evidence that after injury, the newly recruited epithelial cells express antigens characteristic of basal cells. Only after the cells have differentiated into columnar cells do they express antigens characteristic of columnar and ciliated cells. This suggests that basal cells may be the precursor cells for repair after injury, but the possibility that goblet cells can both dedifferentiate and proliferate suggests that goblet cells also could have a role in repair.^{20, 21}

The concept that airway epithelial cells can migrate and thus could be recruited to a site of injury is supported by a body of *in vitro* data. Shoji and colleagues,²² for example, have shown not only

that epithelial cells can migrate, but using the blindwell chamber technique, they have demonstrated that epithelial cells can migrate toward a chemotactic stimulus. The stimulus that drives epithelial cell migration to cover a site of injury in the airways is unknown. One potential candidate mediator, however, is fibronectin, a glycoprotein thought to have important roles in wound repair throughout the body.^{23, 24} Fibronectin is a potent chemotactic stimulus capable of attracting airway epithelial cells.²⁵ Fibronectin, moreover, is a product of epithelial cells.²⁵ Thus the epithelial cells left behind in a wound could produce fibronectin, which could then serve to recruit neighboring cells into the defect.

Several other lines of *in vitro* experimental evidence also support this paracrine concept of epithelial mediated repair in the airway. First, the production of fibronectin by epithelial cells is subject to regulation.²⁶⁻³⁰ An important stimulus is transforming growth factor beta (TGF- β), which stimulates fibronectin production by a number of cell types, including epithelial cells.^{26, 30} Interestingly, epithelial cells in subconfluent cultures are capable of both producing TGF- β and of releasing it in an active form.³¹ Thus it is reasonable to suggest that basal cells that remain at a site of injury could release TGF- β , which could then act in an autocrine or paracrine manner to drive basal cell fibronectin production. This fibronectin could then recruit neighboring epithelial cells to a site of injury.

Other sources of fibronectin at a wound exist. Plasma, for example, contains fibronectin in reasonably large concentrations and with edema, transudation of plasma fibronectin into a wound site will occur.^{23, 24} Cellular derived fibronectin, however, is much more potent than plasma fibronectin as an epithelial cell chemoattractant.²⁵ This suggests that fibronectin derived from epithelial cells could function as a chemoattractant even if plasma leakage occurred at a site of injury. Other cells present in a wound, such as macrophages and fibroblasts, also can produce fibronectin, which could serve as an epithelial cell chemoattractant.^{23, 24}

Chemotaxis is a complex process, and the ability of epithelial cells to mount a repair response likely can be regulated at several levels. For example, inflammatory cell mediators can alter epithelial cell chemotactic responsiveness.^{32, 33} Thus an inflamed mucosa in which phlogistic mediators are present in the local milieu might have a different capacity for repair than a normal epithelium. The

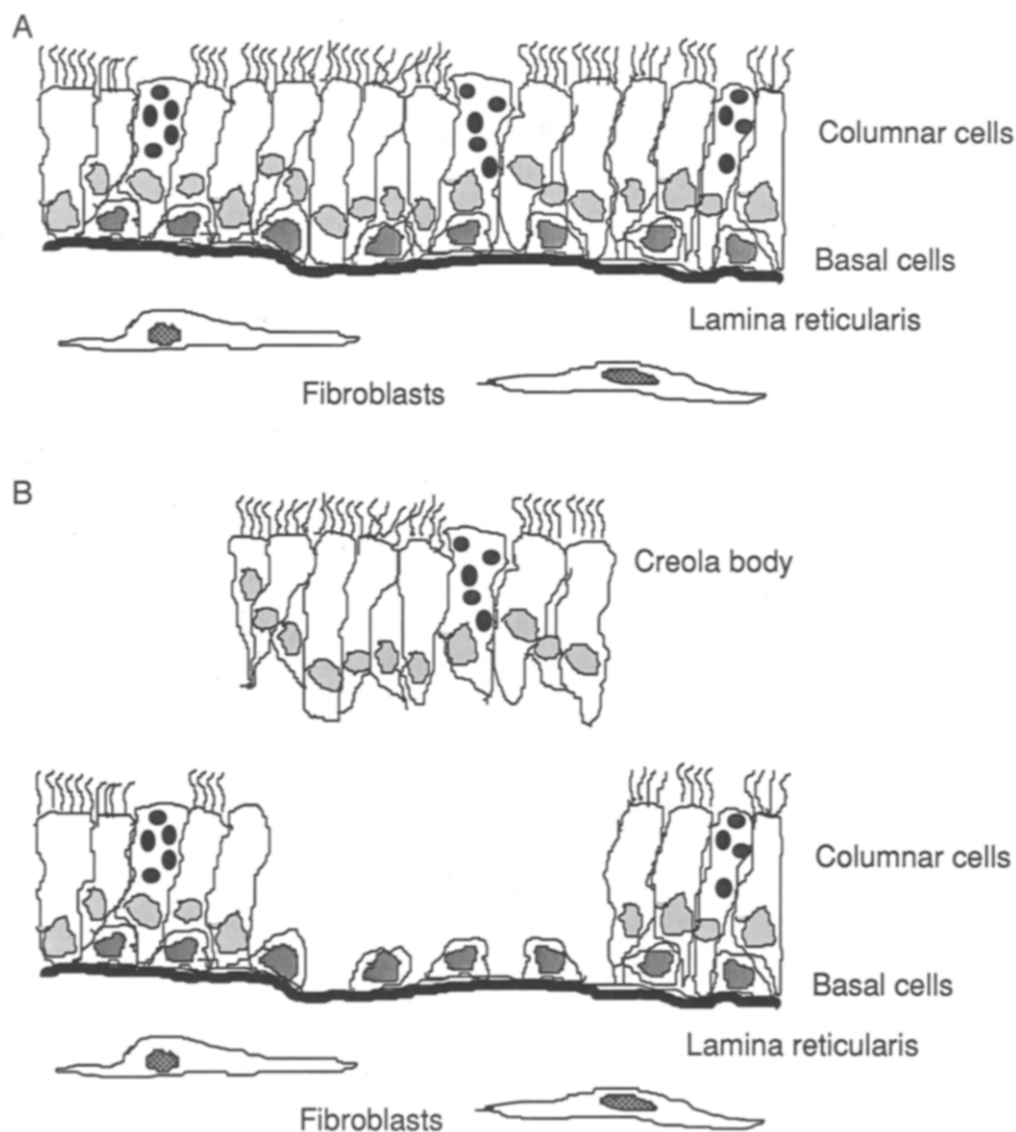


FIG. 1.

chemotactic migration of epithelial cells, moreover, takes place on a surface, and the composition of the surface can alter the chemotactic activity of the cells. Basement membrane components such as laminin and type IV collagen, for example, are excellent surfaces for epithelial cell adhesion but seem to inhibit chemotaxis relative to the interstitial type I collagen.^{34, 35} Thus alteration in the extracellular matrix at a site of injury, an event that is likely to occur if inflammatory cell proteases are released, might also be an important determinant of repair potential in the airway.

As suggested above, chemotactic recruitment of epithelial cells to a site of injury is followed by proliferation of the newly recruited cells. The factors that drive epithelial cell proliferation in this

situation are not yet described. There are, however, several candidate sources for such factors. Mononuclear phagocytes have been suggested to be potent sources of growth factors in other repair processes,³⁶ and factors derived from alveolar macrophages can drive airway epithelial cell proliferation.³⁷ The epithelial cells themselves are also likely sources of growth factors that can function in an autocrine or paracrine manner. In this regard, the epithelial cells can produce both TGF- α , which is believed to stimulate proliferation,³⁸ and TGF- β , which should inhibit proliferation and cause the cells to assume a squamous phenotype.^{31, 39} Fibroblasts also are present in the epithelium and factors derived from these cells are capable of driving epithelial cell proliferation.⁴⁰ Which factors

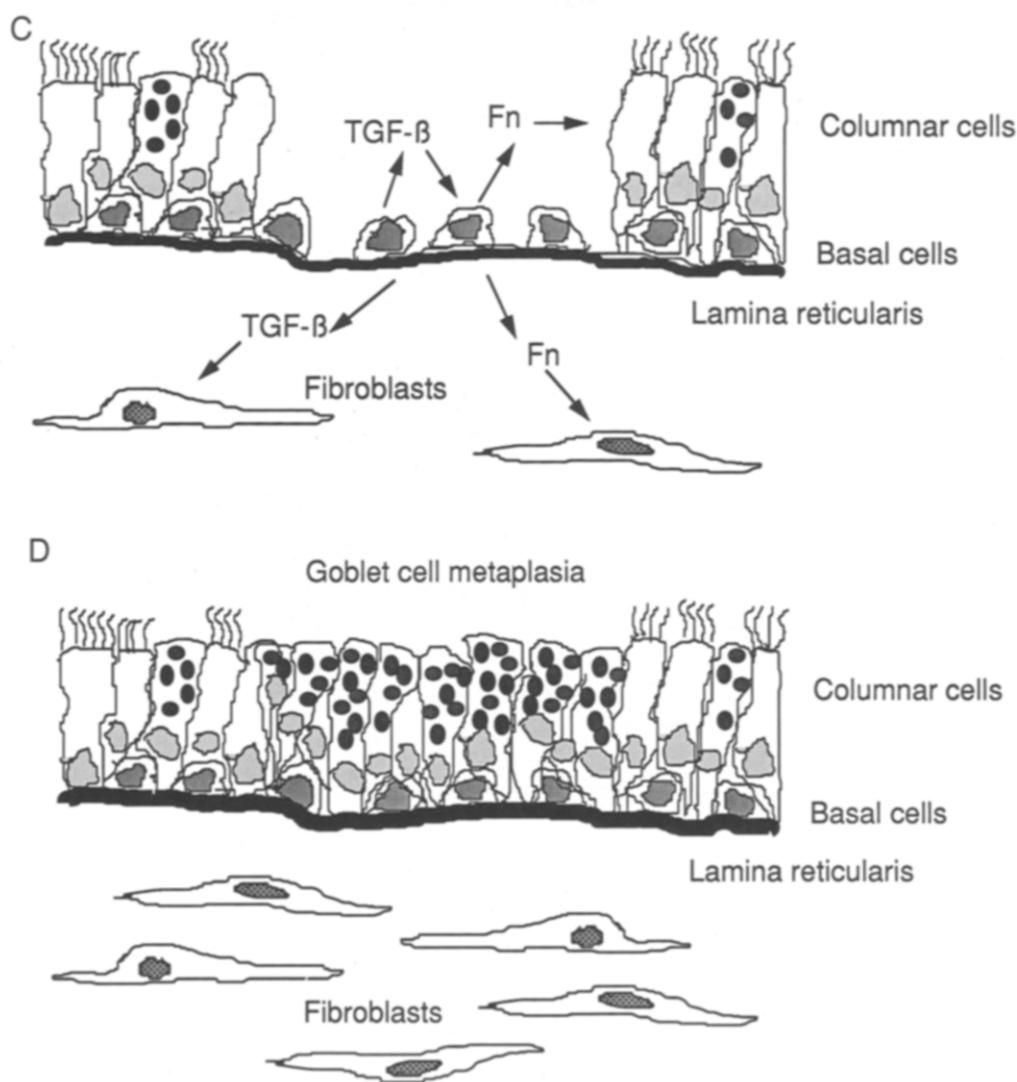


FIG. 1. Cont'd

are responsible for repair in a disease process like asthma will be an important area for future study.

Repair processes in the airway appear to be able to restore the airway architecture to normal after many injuries. Complete restoration, however, is not always the result of these processes. In a series of studies, Snider and colleagues demonstrated the consequences of injury of the airway epithelium in hamsters after injury with the neutrophil protease elastase.⁴¹⁻⁴⁵ Acute exposure was associated with a loss of detectable secretory cells, presumably because elastase induced their degranulation.⁴¹ This acute process was followed by a recovery in the number of visible secretory cells, which 16 days after exposure, had significantly increased.^{42, 43, 45} The phenotype of the secretory cells also was altered with a marked increase in the density of the

granules.⁴¹ These metaplastic changes induced by a single exposure to elastase, moreover, were quite persistent, lasting close to the expected lifetime of the animal.⁴⁴ Other stimuli also can have similar effects.⁴⁶ These results suggest that goblet cell metaplasia develops early in the response to injury and that it can be essentially permanent even without further stimulus. Because goblet cell metaplasia is a regular feature of asthma, the airways of asthmatic patients may thus reflect long-term changes caused by episodic injuries.

In addition to metaplasia of the epithelium, repair after injury can lead to alterations in the mesenchymal structures underlying the airway epithelium. The accumulation of fibroblasts and the connective tissue produced by these cells is a process analogous to scar formation in the skin. It is of interest that airway epithelial cells appear to

be able to drive not only epithelial repair but also mesenchymal cell responses. In this regard, fibronectin derived from epithelial cells is a potent chemotactic stimulus for fibroblasts and for epithelial cells.⁴⁷ Airway epithelial cells also produce mitogens capable of stimulating fibroblast proliferation.^{48, 49} The TGF- β produced by epithelial cells is capable of upregulating fibroblast production of matrix molecules, including type I collagen and fibronectin.⁵⁰ Finally, TGF- β produced by airway epithelial cells can induce fibroblasts to contract a three-dimensional collagen gel in an *in vitro* model of scar contraction.⁵¹ Taken together, these data suggest that airway epithelial cells may be important sources of mediators that could contribute to all aspects of fibrosis and scarring in the airways. These mediators also could alter the function of mesenchymal cells in the airways. Nogami and colleagues,⁵² for example, have shown that TGF- β can induce a loss of β -receptors from cultured airway smooth muscle cells.⁵²

Undoubtedly many other cells present in the airways in a disease such as asthma are also potential sources of mediators that could drive a fibrotic response. It is of interest, however, that airway epithelial cells are potential sources of mediators that can lead either to restoration of normal airway architecture or to the accumulation of fibrous scar tissue. For example, the same mediators, that is, TGF- β and fibronectin, may play a role in the pathogenesis of both processes. What determines whether normal repair with restoration of function will occur after injury or whether scarring will result is undetermined, but presumably the temporal sequence of factors, interrelationships between factors, and the status of both the extracellular matrix and the parenchymal cells available all play a role.

In this regard, inhibitors of the fibrotic process are produced by airway epithelial cells. Prostaglandin E, for example, can inhibit fibroblast chemotaxis,⁵³ proliferation,⁵⁴ collagen production,^{55, 56} and collagen gel contraction.⁵⁷ Prostaglandin E is a major product of airway epithelial cells.^{58, 59} This raises the possibility that the airway epithelium may function to restrict the severity of a fibrotic response after injury. It also raises the possibility that the airway epithelium might play a role in normal airway mesenchymal cell homeostasis by providing an inhibitory stimulus, thus helping to assure airway patency.

Excessive accumulations of connective tissue clearly can compromise lung function. In the most extreme example, obliterative bronchiolitis, plugs

of connective tissue completely obstruct the lumen of the small airways.⁶⁰ Less severe forms of airway fibrosis also can have an important impact on lung function. Although the lesions that contribute to airflow obstruction in chronic obstructive pulmonary disease (COPD) are complex,^{61, 62} peribronchial fibrosis is one of the histologic features that correlates best with airflow obstruction in moderate cases of this disease.⁶³ The small airways of these patients, moreover, are narrowed,^{64, 65} suggesting that scarring has developed around the circumference of the airway and that, as with all scars, the peribronchial fibrotic lesion has contracted, thus narrowing the airway and reducing airflows.

Whereas progressive airflow limitation is well recognized in COPD,^{66, 67} similar processes may take place in asthma. Wagner and colleagues,⁶⁸ for example, demonstrated that asthmatic patients have markedly increased resistance in their peripheral airways, a lesion consistent with narrowing of these airways. In addition, epidemiologic data support this concept. Peat and colleagues,⁶⁹ for example, reported a long-term study of asthmatic patients followed-up for 18 years. Having asthma was a risk factor associated with a decline in lung function twice that of healthy nonsmokers. Several subsequent studies also have suggested that asthma is associated with a risk for an accelerated and progressive loss of lung function.^{70, 71} Thus the long-term prognosis for an asthmatic patient should not be regarded as benign. It seems quite likely that ongoing airway injury leads to alterations in airway structure, which can compromise lung function in a gradual manner over a period of years.

The undoubtedly speculative concept that airway scarring can develop as a consequence of asthma and can, over years, lead to progressive loss of lung function, suggests that this loss of lung function possibly can contribute to the development of COPD. Nevertheless, the question remains whether therapeutic intervention can alter this process. Relatively little data exist to address this question. Several studies have shown that inhaled glucocorticoids can reduce inflammation in the airways,⁷²⁻⁷⁴ and one study has demonstrated that glucocorticoids also can reverse some of the metaplastic changes that characterize asthma.⁷⁴ Whether alterations in the extracellular matrix can be modified is controversial. One study has reported that inhaled glucocorticoid therapy was associated with a decrease in the thickness of the lamina reticularis in patients with asthma.⁷³

Other studies, however, have not been able to detect this potential benefit.^{75,76} Histologic studies of patients with severe inhalation injuries support the theory that remodeling of an apparently scarred airway to restore function is possible. Whether therapeutic interventions can be developed to achieve similar results in patients with asthma or COPD is a crucial question to be answered.

NORMAL EPITHELIUM

The normal airway epithelium (Fig. 1A) is lined by a complex layer of epithelial cells.^{77,78} In the proximal airways it is pseudostratified, meaning that all cells are believed to have contact with the basement membrane, although several layers of nuclei may be present. Most of the basement membrane surface is covered by basal cells, which anchor the epithelium via hemidesmosomes.⁷⁹ The majority of cells that reach the airway lumen are ciliated cells, although smaller numbers of secretory cells (goblet cells) also are present. These columnar cells are attached to each other and to the basal cells by desmosomes. Near the apical lumen the columnar cells also possess tight junctions, thus creating the epithelial barrier.⁸⁰ The entire epithelium rests on a thin true basement membrane, which in turn is underlaid by a lamina reticularis comprised of interstitial connective tissue. The subepithelium contains additional connective tissue and fibroblasts.

EPITHELIAL INJURY IN ASTHMA

Epithelial injury regularly occurs in asthma and can result in desquamation of epithelial cells (Fig. 1B). Frequently sheets of columnar cells are detached as aggregates, resulting in the formation of creola bodies.¹³ The epithelial defect can alter airway function in asthma by a variety of mechanisms.^{78,81,82} First, the epithelial barrier is disrupted, permitting both ready absorption of environmental agents and leakage of plasma components into the airway lumen. Airway nerves also may become exposed, predisposing the airway to reflex bronchoconstriction. Second, epithelial cells can inhibit airway reactivity both by releasing inhibitors and by degrading active mediators. Loss of epithelial cells removes this restraining effect of the airway epithelium. Third, cells left behind in the airway may be able to release mediators that can induce reactivity of the airways.

RESPONSE OF THE EPITHELIUM TO INJURY

Injury of the epithelium initiates a complex cascade of responses (Fig. 1C). Epithelial cells are activated to release a variety of mediators, includ-

ing TGF- β and fibronectin, which can serve to recruit and activate both neighboring epithelial cells and subjacent mesenchymal cells. Epithelial cells also can release a variety of mediators that can recruit and activate inflammatory cells (not shown in Fig. 1C) and can, moreover, respond to mediators released by inflammatory cells.^{78,83,84} The net effect on epithelial repair likely depends on mediators released by many cell types in the airway. Under some circumstances, normal epithelial architecture can be restored (Fig. 1A). Under other conditions, repair can result in chronic pathologic alterations of airway structures (Fig. 1D).

REPAIR WITH ALTERED ARCHITECTURE

Repair of the airway epithelium does not necessarily restore the epithelial architecture to normal (Fig. 1D). The normal ciliated epithelial cells can be replaced by goblet cells or even squamous cells (not shown in Fig. 1D). In addition, increased numbers of mesenchymal cells can accumulate in the subepithelial layer together with the connective tissue matrix produced by these cells. It is likely that these cells are responsible for the thickened lamina reticularis that characterizes asthma.^{10,85} These changes in connective tissue may be associated with narrowing of the airways and compromise of airflow.

In summary, the airway in asthmatic patients is subject to injury. As a result, repair processes are initiated. In most individuals, most injuries are repaired with restoration of normal tissue architecture and function. In some cases, however, structural alterations result, sometimes with compromise of lung function. Currently available data suggest that many cells in the airway play roles in the repair process. The airway epithelial cell seems to play a particularly important role, functioning both as a source of mediators of repair and as a target for those mediators. As abnormal repair appears to be responsible for many aspects of chronic disease, the potential to modulate repair processes represents an important therapeutic target.

REFERENCES

1. Sheffer AL. International consensus report on diagnosis and treatment of asthma. *Eur Respir J* 1992;5:601-41.
2. Glynn A. Bronchial biopsy in chronic bronchitis and asthma. *Thorax* 1960;15:132-41.
3. Thurlbeck WM, Henderson JA, Fraser RG, Bates DV. Chronic obstructive lung disease. A comparison between clinical, roentgenologic, functional and morphologic criteria in chronic bronchitis, emphysema, asthma and bronchiectasis. *Medicine* 1970;49:81-145.

4. Takizawa T, Thurlbeck WM. Muscle and mucous gland size in the major bronchi of patients with chronic bronchitis, asthma, and asthmatic bronchitis. *Am Rev Respir Dis* 1971;104:331-6.
5. Spencer H. Bronchial asthma. In: *Pathology of the Lung*. Oxford: Pergamon Press, 1968:715-20.
6. Woolcock AJ. Asthma. In: Murray JF, Nadel JA, eds. *Textbook of Respiratory Medicine* (vol. 2). Philadelphia: W.B. Saunders, 1994:1288-1330.
7. Cutz E, Levison H, Cooper DM. Ultrastructure of airways in children with asthma. *Histopathology* 1978;2:407-21.
8. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. *Am Rev Respir Dis* 1989;140:1745-53.
9. Dunnill MS, Masaralla GR, Anderson JA. A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmaticus, in chronic bronchitis, and in emphysema. *Thorax* 1969;24:176-9.
10. Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;i:520-4.
11. Naylor B. The shedding of the mucosa of the bronchial tree in asthma. *Thorax* 1962;17:69-72.
12. Sanerkin NG, Evans DMD. The sputum in bronchial asthma. *J Pathol Bacteriol* 1965;89:535-541.
13. Naylor B, Railey C. A pitfall in the cytodiagnosis of sputum of asthmatics. *J Clin Pathol* 1964;17:84-9.
14. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989;139:806-17.
15. McIntosh K, Ellis EF, Hoffman LS, Lybass TG, Eller JJ, Fulginiti VA. The association of viral and bacterial infections with exacerbations of wheezing in your asthmatic children. *J Pediatr* 1973;82:578-90.
16. Minor TE, Dick EC, Baker JW, Oullette JJ, Cohen M, Reed CE. Rhinovirus and influenza type A infections as precipitants of asthma. *Am Rev Resp Dis* 1976;113:149-53.
17. Lane BP, Gordon R. Regeneration of rat tracheal epithelium after mechanical injury. *Proceedings of the Society for Experimental Biology and Medicine* 1974;145:1139-44.
18. Keenan KP, Combs JW, McDowell EM. Regeneration of hamster tracheal epithelium after mechanical injury. *Virchows Arch* 1982;41:193-214.
19. Shimizu T, Nishihara M, Kawaguchi S, Sakakura Y. Expression of phenotypic markers during regeneration of rat tracheal epithelium following mechanical injury. *Am J Respir Cell Mol Biol* 1994;11:85-94.
20. Liu JY, Nettesheim P, Randell SH. Growth and differentiation of tracheal epithelial progenitor cells. *Am J Physiol* 1994;266:L296-L307.
21. Johnson NF, Hubbs AF. Epithelial progenitor cells in the rat trachea. *Am J Respir Cell Mol Biol* 1990;3:579-85.
22. Shoji S, Ertl RF, Linder J, Koizumi S, Duckworth WC, Rennard SI. Bronchial epithelial cells respond to insulin and insulin-like growth factor-I as a chemoattractant. *Am J Respir Cell Mol Biol* 1990;2:553-7.
23. Hynes RO, Yamada KA. Fibronectins: multifunctional modular glycoproteins. *J Cell Biol* 1982;95:369-377.
24. Roman J, McDonald JA. Fibronectins. In: Crystal RG, West JB, eds. *The Lung: Scientific Foundations* (vol. 1). New York: Raven Press, 1991:399-411.
25. Shoji S, Ertl RF, Linder J, Romberger DJ, Rennard SI. Bronchial epithelial cells produce chemotactic activity for bronchial epithelial cells: Possible role for fibronectin in airway repair. *Am Rev Respir Dis* 1990;141:218-25.
26. Romberger DJ, Beckmann JD, Claassen L, Ertl RF, Rennard SI. Modulation of fibronectin production of bovine bronchial epithelial cells by transforming growth factor-beta. *Am J Respir Cell Mol Biol* 1992;7:149-55.
27. Romberger DJ, Claassen L, Toews M, Beckmann J, Rennard SI. Isoproterenol alters the induction of fibronectin by TGF-beta from bronchial epithelial cells [Abstract]. *Am Rev Respir Dis* 1991;143:532.
28. Adachi Y, Mio T, Striz I, et al. Lipopolysaccharide (LPS) increases fibronectin production and release from cultured lung fibroblasts partially through proteolytic activity. *J Lab Clin Med* 1996;127:448-55.
29. Romberger DJ, Pladsen P, Claassen L, Yoshida M, Beckmann JD, Rennard SI. Insulin modulation of bronchial epithelial cell fibronectin in vitro. *Am J Physiol* 1995;268:L230-8.
30. Wang A, Cohen DS, Palmer E, Sheppard D. Polarized regulation of fibronectin secretion and alternative splicing by transforming growth factor. *J Biol Chem* 1991;266:15598-601.
31. Sacco O, Romberger D, Rizzino A, Beckmann J, Rennard SI, Spurzem JR. Spontaneous production of transforming growth factor beta 2 by primary cultures of bronchial epithelial cells: effects on cell behavior in vitro. *J Clin Invest* 1992;90:1379-85.
32. Ito H, Romberger DJ, Rennard SI, Spurzem JR. TNF-alpha enhances bronchial epithelial cell migration and attachment to fibronectin. *Am Rev Respir Dis* 1993;147:A46.
33. Ito H, Rennard SI, Spurzem JR. Mononuclear cell conditioned medium enhances bronchial epithelial cell migration but inhibits attachment fibronectin. *J Lab Clin Med* (in press).
34. Rickard KA, Taylor J, Rennard SI, Spurzem JR. Migration of bovine bronchial epithelial cells to extracellular matrix components. *Am J Respir Cell Mol Biol* 1993;8:63-8.
35. Isliker H, Bing DH, Hynes RO. Interactions of fibronectin with C1q, a subcomponent of the first component of complement. *The Immune System* 1981;2:231-8.
36. Crystal RG. Alveolar macrophages. In: Crystal RG, West JB, eds. *The Lung: Scientific Foundations*, vol 1. New York: Raven Press, 1991:527-38.
37. Takizawa H, Beckmann J, Shoji S, et al. Pulmonary macrophages can stimulate cell growth of bovine bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1990;2:245-55.
38. Ferriola PC, Robertson AT, Rusnak DW, Diaugustine R, Nettesheim P. Epidermal growth factor dependence and TGF alpha autocrine growth regulation in primary rat tracheal epithelial cells. *J Cell Physiol* 1992;152:302-9.
39. Pfeifer MA, Lechner JF, Masui T, Reddel RR, Mark GE, Harris CC. Control of growth and squamous differentiation in normal human bronchial epithelial cells by chemical and biological modifiers and transferred genes. *Environ Health Perspect* 1989;80:209-20.
40. Shoji S, Rickard KA, Takizawa H, Ertl RF, Linder J, Rennard SI. Lung fibroblasts produce growth stimulatory activity for bronchial epithelial cells. *Am Rev Respir Dis* 1990;141:433-9.
41. Breuer R, Christensen TG, Lucey EC, Stone PJ, Snider GL. An ultrastructural morphometric analysis of elastase-treated hamster bronchi shows discharge followed by progressive accumulation of secretory granules. *Am Rev Respir Dis* 1987;136:698-703.

42. Christensen TG, Breuer R, Hornstra LJ, Lucey EC, Stone PJ, Snider GL. An ultrastructural study of the response of hamster bronchial epithelium to human neutrophil elastase. *Exp Lung Res* 1987;13:279-97.
43. Breuer R, Christensen TG, Lucey EC, Stone PJ, Snider GL. Quantitative study of secretory cell metaplasia induced by human neutrophil elastase in the large bronchi of hamsters. *J Lab Clin Med* 1985;105:635-40.
44. Christensen TG, Korthy AL, Snider GL, Hayes JA. Irreversible bronchial goblet cell metaplasia in hamsters with elastase-induced panacinar emphysema. *J Clin Invest* 1977;59:397-404.
45. Snider GL, Lucey EC, Christensen TG, et al. Emphysema and bronchial secretory cell metaplasia induced in hamsters by human neutrophil products. *Am Rev Respir Dis* 1984;129:155-60.
46. Christensen TG, Lucey EC, Breuer R, Snider GL. Acid-induced secretory cell metaplasia in hamster bronchi. *Environ Res* 1988;45:78-90.
47. Shoji S, Rickard KA, Ertl RF, Robbins RA, Linder J, Rennard SI. Bronchial epithelial cells produce lung fibroblast chemotactic factor: Fibronectin. *Am J Respir Cell Mol Biol* 1989;1:13-20.
48. Nakamura Y, Tate L, Ertl RF, et al. Bronchial epithelial cells regulate fibroblast proliferation. *Am J Physiol* 1995;269:L377-L87.
49. Cambrey AD, Kwon OJ, Gray AJ, et al. Insulin-like growth factor I is a major fibroblast mitogen produced by primary cultures of human airway epithelial cells. *Clin Sci (Colch)* 1995;89:611-7.
50. Kawamoto M, Romberger DJ, Nakamura Y, et al. Modulation of fibroblast type I collagen and fibronectin production by bovine bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1995;12:425-33.
51. Mio T, Adachi Y, Romberger DJ, et al. Human bronchial epithelial cells modulate collagen gel contraction by fibroblasts [Abstract]. *Am J Respir Crit Care Med* 1995;151:561.
52. Nogami M, Romberger DJ, Rennard SI, Toews ML. TGF-beta modulates beta-adrenergic receptor number and function in cultured human tracheal smooth muscle cells. *Am J Physiol* 1994;266:L187-L91.
53. Ertl RF, Valenti V, Spurzem JR, et al. Prostaglandin E inhibits fibroblast recruitment [Abstract]. *Am Rev Respir Dis* 1992;145:19.
54. Bitterman PB, Wewers MD, Rennard SI, Adelberg S, Crystal RG. Modulation of alveolar macrophage-driven fibroblast proliferation by alternative macrophage mediators. *J Clin Invest* 1986;77:700-8.
55. Bienkowski RS, Gotkin MG. Control of collagen deposition in mammalian lung. *Proc Soc Exp Biol Med* 1995;209:118-40.
56. Fine A, Poliks CF, Donahue LP, Smith BD, Goldstein RH. The differential effect of prostaglandin E2 on transforming growth factor-beta and insulin-induced collagen formation in lung fibroblasts. *J Biol Chem* 1989;264:16988-91.
57. Ehrlich HP, Wyler DJ. Fibroblast contraction of collagen lattices in vitro: inhibition by chronic inflammatory cell mediators. *J Cell Physiol* 1983;116:345-51.
58. Leikauf GD, Driscoll KE, Wey HE. Ozone-induced augmentation of eicosanoid metabolism in epithelial cells from bovine trachea. *Am Rev Respir Dis* 1988;137:435-42.
59. Leikauf GD, Ueki IF, Nadel JA, Widdicombe JH. Bradykinin stimulates CI selection and prostaglandin release by canine tracheal epithelium. *Am J Physiol* 1985;248:F48-F55.
60. Myers JL, Colby TV. Pathologic manifestations of bronchiolitis, constrictive bronchiolitis, cryptogenic organizing pneumonia and diffuse panbronchiolitis. *Clin Chest Med* 1993;14:611-22.
61. Niewoehner DE, Sobonya RE. Structure-function correlations in chronic obstructive pulmonary disease. In: Baum GL, Wolinsky E, eds. *Textbook of Pulmonary Diseases* (vol. 2). Boston: Little, Brown and Company, 1993:973-93.
62. Sobonya RE. The morphologic basis for diseases of airways obstruction. In: Baum GE, Wolinsky E, eds. *Textbook of Pulmonary Diseases* (vol. 2). Boston: Little, Brown and Company, 1994:955-71.
63. Cosio M, Ghezzi H, Hogg JC, et al. The relations between structural changes in small airways and pulmonary function tests. *N Engl J Med* 1978;298:1277-81.
64. Matsuba K, Wright JL, Wiggs BR, Pare PD, Hogg JC. The changes in airways structure associated with reduced forced expiratory volume in one second. *Eur Respir J* 1989;2:834-9.
65. Kuwano K, Bosken CH, Pare PD, Bai TR, Wiggs BR, Hogg JC. Small airways dimensions in asthma and in chronic obstructive pulmonary disease. *Ann Rev Respir Dis* 1993;148:1220-5.
66. Fletcher C, Peto R, Tinker C, Speizer F. The natural history of chronic bronchitis and emphysema. New York: Oxford University Press, 1976:1-272.
67. Snider GL, Faling LJ, Rennard SI. Chronic bronchitis and emphysema. In: Murray JF, Nadel JA, eds. *Textbook of Respiratory Medicine*. Philadelphia: W.B. Saunders, 1994;2:1331-97.
68. Wagner EM, Liu MC, Weinmann GG, Permutt S, Bleeker ER. Peripheral lung resistance in normal and asthmatic subjects. *Am Rev Respir Dis* 1990;141:584-8.
69. Peat JK, Woolcock AJ, Cullen K. Rate of decline of lung function in subjects with asthma. *Eur J Respir Dis* 1987;70:171-9.
70. Sherman CB, Xu X, Speizer FE, Ferris BG, Weiss ST, Dockery DW. Longitudinal lung function decline in subjects with respiratory symptoms. *Am Rev Respir Dis* 1992;146:855-9.
71. Krzyzanowski M, Camilli AE, Lebowitz MD. Relationships between pulmonary function and changes in chronic respiratory symptoms. *Chest* 1990;98:62-70.
72. Burke C, Power CK, Norris A, Condez A, Schmekel B, Poulter LW. Lung function and immunopathological changes after inhaled corticosteroid therapy in asthma. *Eur Respir J* 1992;5:73-9.
73. Trigg CJ, Manolitsas ND, Wang J, et al. Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. *Am J Respir Crit Care Med* 1994;150:17-22.
74. Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide and a β_2 -agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial. *J Allergy Clin Immunol* 1992;90:32-42.
75. Laitinen A, Altraja A, Linden M, et al. Treatment with inhaled budesonide and tenascin expression in bronchial mucosa of allergic asthmatics [Abstract]. *Am J Respir Crit Care Med* 1994;149:942.
76. Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson S-A. Effects of treatment on airway inflamma-

- tion and thickening of basement membrane reticular collagen in asthma. *Am Rev Respir Dis* 1992;145:890-9.
77. Breeze RG, Wheeldon EB. The cells of the pulmonary airways. *Am Rev Respir Dis* 1977;116:705-77.
78. Rennard SI, Beckmann JD, Robbins RA. Biology of airway epithelial cells. In: Crystal RG, West JB, eds. *The lung: scientific foundations*. New York: Raven Press, 1990:157-67.
79. Evans MJ, Plopper CG. The role of basal cells in adhesion of columnar epithelium to airway basement membrane. *Am Rev Respir Dis* 1988;138:481-3.
80. Schneeberger EE. Airway and alveolar epithelial cell junctions. In: Crystal RG, West JB, eds. *The lung: scientific foundations*. New York: Raven Press, 1990:157-67.
81. Shelhamer JH, Levine SJ, Jacoby DB, Kaliner MA, Rennard SI. Airway inflammation. *Ann Intern Med* 1995;123:288-304.
82. Rennard SI, West WW, Robbins RA. Epithelial cells in host defense. In: Burnett D, Stookey RA, eds. *Pulmonary Defences*. Chichester: John Wiley & Sons, Ltd (in press).
83. Thompson AB, Robbins RA, Romberger DJ, et al. Immunological functions of the pulmonary epithelium. *Eur Respir J* 1995;8:127-49.
84. Rennard SI, Von Essen S, Thompson A, et al. Clinical implications of airways inflammation. In: Marone G, Pozzi E, Rennard SI, eds. *Basophils, mast cells and airway epithelial cells*. Milan: Masson, 1994:229-34.
85. Brewster CE, Horwarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1990;3:507-11.