

# Remodeling of asthmatic airways by glucocorticosteroids

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Airway epithelial damage and chronic inflammation are the main contributory factors in the development of asthma. Until bronchoscopic techniques were developed, clear evidence of the inflammation was relatively unknown. Today, with electron microscopy of bronchial biopsy specimens or immunohistochemistry studies, a definitive picture emerges of the cellular changes associated with the chronic inflammation of asthma, as well as evidence of the vascular disturbances that characterize this disease.<sup>1-8</sup>

Recent studies have also shown that glucocorticoids ameliorate airways mucosal and epithelial damage by normalizing different cell types and by increasing the number of intraepithelial nerves and decreasing the number of endothelial gaps in the postcapillary venules.<sup>9</sup> Glucocorticoids may have an effect on the accumulation and thickening of basement membrane, thus improving intercellular communication and preventing asthma recurrences (Fig. 1).

Although it is known that glucocorticoid treatment reduces the damage caused by asthma on airways mucosa and epithelium, it is difficult to envision the impact of these drugs on remodeling of asthmatic airways without current bronchoscopic techniques. This article presents an outline of airways development and the effect of glucocorticosteroids on the structural and cellular changes that occur in asthma.

## AIRWAYS DEVELOPMENT

Bronchial epithelium is endodermal in origin, whereas the lamina propria is of mesenchymal origin. Therefore on the basis of these observations, it can be assumed that the extracellular matrix pattern is mesenchymal in origin (Fig. 2).

The extracellular matrix is composed of a mixture of macromolecules and fibrous proteins.<sup>10</sup> At present it is known that many ECM proteins, proteoglycans, and the expression of cellular receptors are needed for normal airway branching morphogenesis.<sup>11</sup> Laminins and type III collagen are important to airway development. The addition of antilaminin antibodies to embryonic lung cultures leads to decreased tube formation, terminal branching, and epithelial cell mitosis.<sup>11, 12</sup> Also, neuropeptides such as bombesin-like peptides (e.g., gastrin-releasing peptide), which increase cleft formation and neutral endopeptidase, which leads to degradation of peptides, may be important during airway morphogenesis.

The development and repair mechanisms of the airways are similar in that the process of wound healing recapitulates the main events commonly associated with embryogenesis.<sup>13</sup> During development of the airways, the extracellular matrix regulates the differentiation, organization, and function of epithelial cells, in particular cell shape, cell polarity, and differentiation of function. At the same time, epithelial cells regulate the production of the extracellular matrix.<sup>11</sup>

## STRUCTURE OF ASTHMATIC AIRWAYS

Fig. 3 illustrates the different events during development and the repair systems that occur in airways. It is important, however, to understand the components of the extracellular matrix and basement membrane to grasp the changes that occur during asthma.

### Extracellular matrix

The extracellular matrix is composed of glycosaminoglycan and proteoglycan macromolecules that form a highly hydrated, gel-like substance in which fibrous proteins are embedded. These fibrous proteins may be structural in type, such as collagen and elastin, or adhesive, such as fibronectin and laminin.<sup>10</sup> Type IV collagen, fibronectin, and laminin are produced by bronchial epithelial cells.<sup>14</sup> Laminin and fibronectin can interact with

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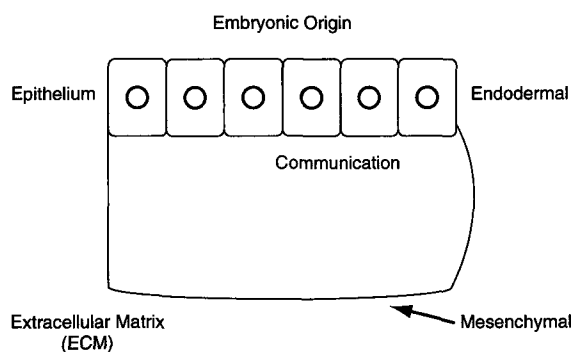


**FIG. 1.** Airway epithelium has endodermal and matrix mesenchymal origin during embryogenesis. Epithelial cells and matrix have interactions mediated by cell surface receptors such as *integrins*.

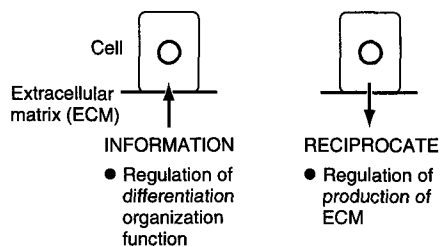


**FIG. 2.** Components of extracellular matrix interact with cell surface molecules such as different types of integrin receptors and proteoglycans. These interactions lead epithelial cells to reciprocate by inducing signal transduction cascades in the cell, altering cellular responses.

the collagenous structures of the extracellular matrix.<sup>10-12</sup>

### Basement membranes

Basement membranes are thin layers of specialized extracellular matrix that form a supporting structure for epithelial cell growth.<sup>10</sup> It has recently been suggested that basement membranes provide not only mechanical support but also influence cellular behavior.<sup>15</sup> Somewhat different from the surrounding extracellular matrix, the major molecular components of basement membranes are type IV collagen, laminin/entactin/nidogen complexes, and proteoglycans.<sup>15</sup>

### Epithelial changes during asthmatic insult

Fig. 4 depicts the predominant changes in asthmatic airways. These are an abnormal airway epithelium, which is characterized by a greater-than-normal ratio of goblet-to-ciliated epithelial cells, a thickening of the basement membrane (Fig. 5),<sup>16</sup>

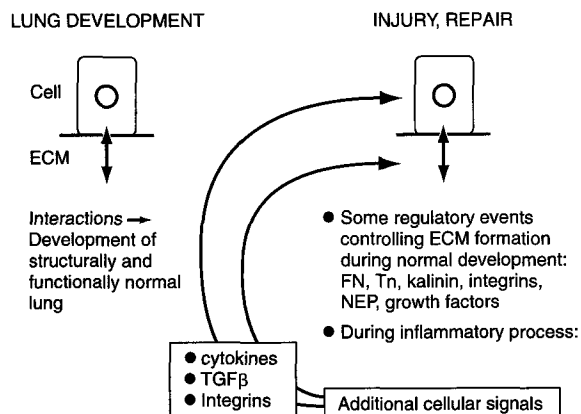


**FIG. 3.** Interactions between epithelial cells and extracellular matrix are important during normal morphogenesis, as shown on left. Similar mechanisms are involved during injury and repair processes, as shown on right. During inflammation, major events commonly associated with embryogenesis may be activated but are modified by mediators released from inflammatory cells and injured tissue.

and an increase in inflammatory cell counts (e.g., mast cells, eosinophils, and lymphocytes).<sup>10</sup> The difference between events during lung development and the repair response to injury is that during the repair response, these events listed occur in concert with the inflammatory process, which includes the release of cytokines and transforming growth factor  $\beta$ .<sup>17</sup> These interactive inflammatory mediators appear to work together, leading to the cellular damage that is often seen in asthmatic patients even with mild disease.

### ANTIINFLAMMATORY EFFECTS OF CORTICOSTEROIDS

Corticosteroid treatment brings about abatement of the inflammatory process. The exact mechanism is not known, but it is thought that corticosteroids diffuse across the cell membrane and bind to specific receptors within the cytoplasm.<sup>9</sup> Several studies have documented the normalizing effect of inhaled corticosteroids on airway inflammation. In earlier studies Haahtela et al.<sup>18, 19</sup> undertook a controlled clinical trial comparing the effect of inhaled budesonide and an inhaled  $\beta_2$ -agonist, terbutaline, on clinical symptoms and lung function in adult patients with newly diagnosed asthma. As a part of that study, biopsy specimens were taken before the study, during a 3-month treatment period with either drug, and after the treatment period and then analyzed by electron microscopy.<sup>9</sup>

Great clinical improvement occurred in the group taking inhaled budesonide. In addition, bi-

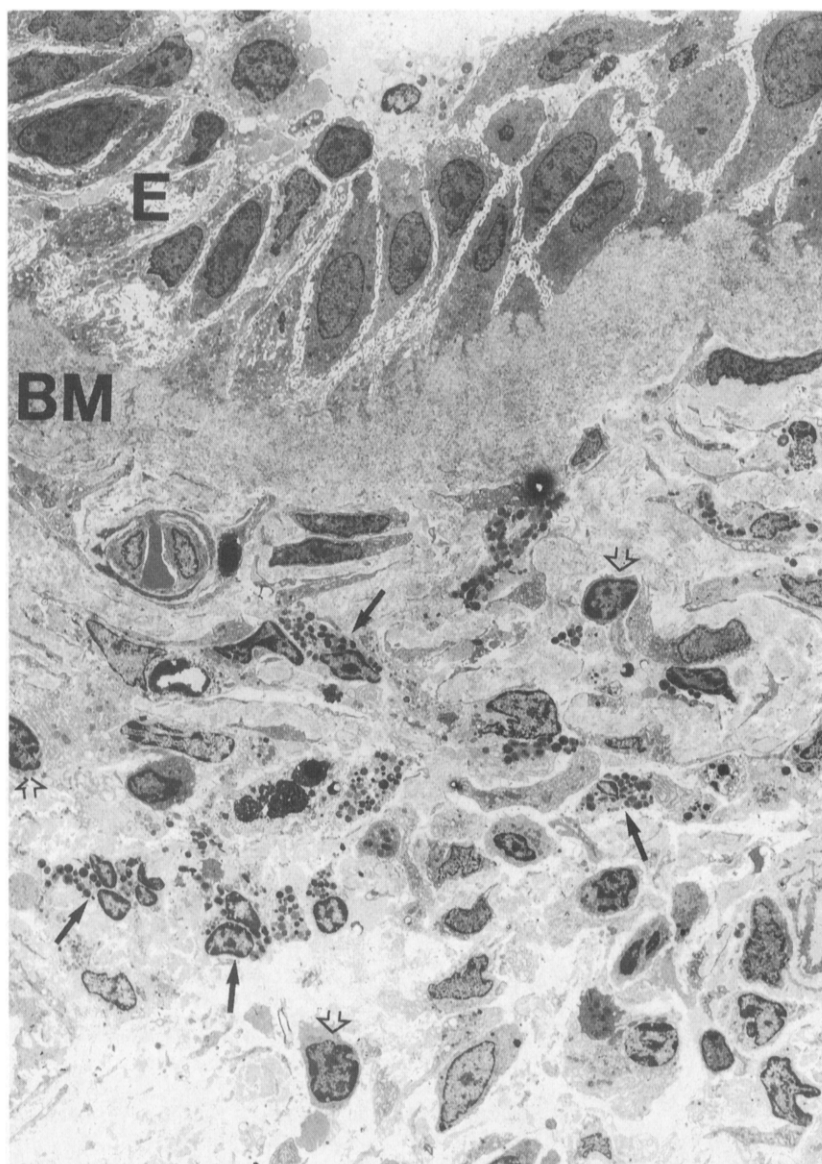


**FIG. 4.** Low-power transmission electron micrograph. Bronchial biopsy specimen from an asthmatic patient having asthma for less than a year. Figure shows damaged airway epithelium (*E*) and a thickened subepithelial basement membrane (*BM*). A strong inflammatory reaction is seen in lamina propria with eosinophils (*black arrows*) and lymphocytes (*open arrows*). (Original magnification  $\times 2000$ .)

opsy findings in the budesonide-treated group showed a reconstruction of epithelial structure, in particular an increase in the ciliated-to-goblet cell ratio from 2:1 before the study to 6:1. Moreover, normalization of epithelial nerves and a decreased number of gaps in postcapillary venules were noted. These findings were not observed in the terbutaline-treated group.

In a recent study<sup>20</sup> the effects of budesonide, 400  $\mu\text{g}$  twice daily, or placebo on basement membrane thickness and composition were compared in pa-

tients with seasonal birch pollen asthma. Biopsy specimens were taken from either budesonide-treated or placebo patients before the season and after a 5- to 6-week treatment period. The extracellular matrix proteins tenascin (Fig. 6) and collagen type III was analyzed by calculating the amount of immunofluorescence staining in each biopsy sample with use of a computerized AutoCad program. In this study there was a significant decrease in the amount of tenascin found in budesonide-treated patients after treatment compared

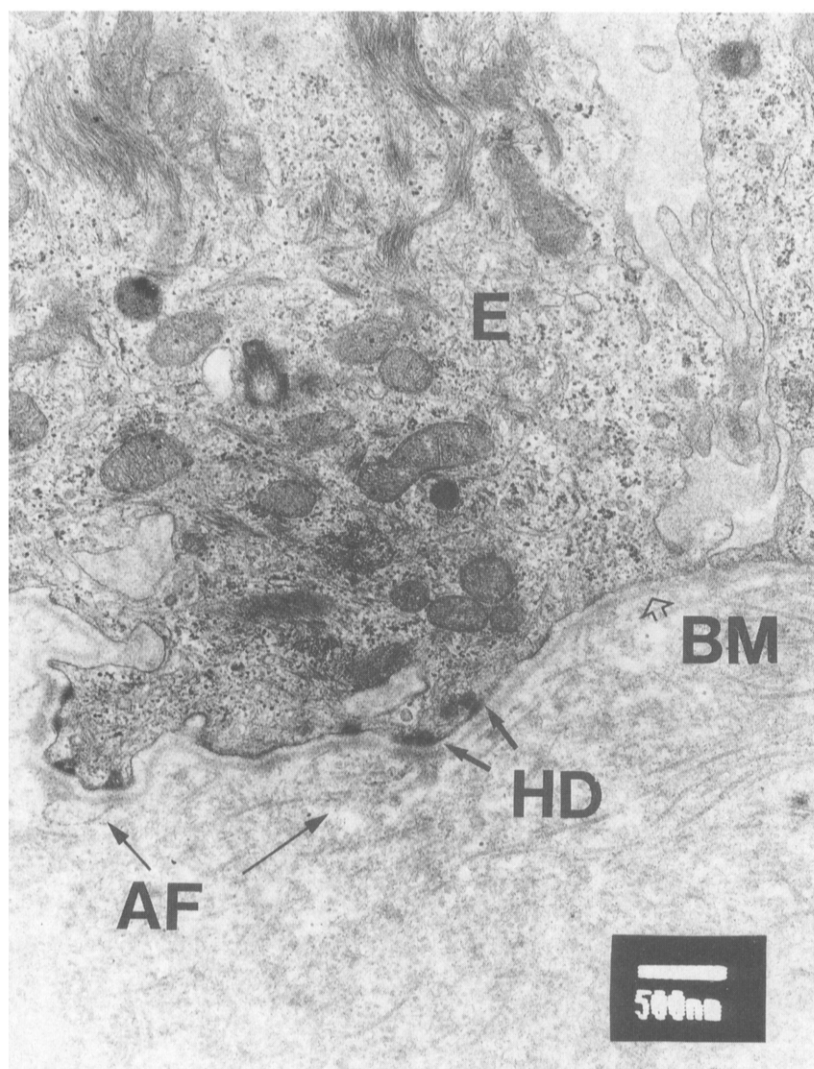


**FIG. 5.** Transmission electron micrograph. Biopsy specimen is from an asthmatic patient. Figure shows a thickened subepithelial basement membrane (*BM*). Basal cells possess hemidesmosomes (*HD*) by which they adhere to superficial part of basement membrane. Anchoring filaments (*AF*) form part of adhesion complex. Bar = 500 nm. (Original magnification  $\times 10,000$ .)

with patients receiving placebo. There were no significant differences in amounts of collagen found in either treatment group.

Trigg et al.<sup>21</sup> have reported the effects on collagen III in the airway basement membrane with twice-daily treatment of beclomethasone dipropionate (1000  $\mu\text{g/day}$ ) over a 4-month period. In this study biopsy specimens were taken from patients receiving either beclomethasone ( $n = 13$ ) or placebo ( $n = 12$ ) and analyzed for inflammatory cell counts and collagens in the basement membrane. After the treatment period, biopsy studies in the beclomethasone-treated group showed, in addition to collagen III, a significant reduction in the num-

ber of mast cells and reductions in the numbers of total eosinophils and activated eosinophils.

It has been suggested that glucocorticoids modulate cell-matrix interactions by influencing increased expression of extracellular matrix components such as tenascin, thereby affecting a down-regulation of inflammation. It is still not clear, however, whether inhaled corticosteroids affect the extracellular matrix specifically or indirectly. Ekblom et al.<sup>22</sup> determined that in cell culture dexamethasone had a direct effect on tenascin but not on laminin. This result suggested the possibility of a direct and specific effect by corticosteroids.



**FIG. 6.** Light microscopy. Immunofluorescence technique using monoclonal antibody against tenascin (*Tn*). Figure shows strong *Tn* immunoreactivity in airway subepithelial basement membrane area. Some immunoreactivity is seen in smooth muscle.

## DISCUSSION AND CONCLUSIONS

The presence or absence of epithelial damage is the main difference in patients with asthma compared with control subjects. We believe that the communication between the different cell types and extracellular matrix proteins is a central determinant of the disease process.

It is also hypothesized that altered communication between cells can occur as a result of varying ratios in cell types (e.g., increased number of goblet-to-ciliated cells) or the presence of different cell receptors and secretory functions (e.g., neuropeptide or neutral endopeptidase), thereby potentiating increased mucous production and changes in basement membrane and/or extracellular matrix components. It is now known that increased tenascin, collagens, and fibronectin result in accumulation and thickening of basement membrane, which further alters communication between epithelial and lamina propria cells.

Glucocorticosteroids have a normalizing effect on different cell types and extracellular matrix components. Glucocorticosteroids may also reduce the thickening of basement membrane through down-regulation of inflammation, thereby influencing better communication on a cellular level and a resultant prevention of asthma.

## REFERENCES

1. Lundgren R, Söderberg M, Horstedt P, Stenling R. Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. *Eur Respir J* 1988;1:883-9.
2. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 1985;131:599-606.
3. 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.
4. Jeffery PK, Wardlaw AJ, Nelson Fiona C, Collins JV, Kay AB. Bronchial biopsies in asthma. *Am Rev Respir Dis* 1990;140:1745-53.
5. Azzawi M, Bradley B, Jeffery PK, et al. Identification of activated T-lymphocytes and eosinophils in bronchial biopsies in stable atopic asthmatics. *Am Rev Respir Dis* 1990;142:1407-13.
6. Bousquet J, Chané P, Lacoste JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
7. Djukanovic R, Wilson JW, Britten KM, et al. Quantification of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. *Am Rev Respir Dis* 1990;142:863-71.
8. Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson S-A. Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma: a quantitative light and electron microscopic study. *Am Rev Respir Dis* 1992;145:890-9.
9. Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a  $\beta_2$ -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.
10. Laitinen A, Laitinen LA. Airway morphology: epithelium/basement membrane. *Am Rev Respir Dis* 1994;150:S14-S7.
11. McGowan SE. Extracellular matrix and the regulation of lung development and repair. *FASEB J* 1992;6:2895-904.

12. Adams JC, Watt FM. Regulation of development and differentiation by the extracellular matrix. *Development* 1993;117:1183-98.
13. Raghow R. The role of extracellular matrix in postinflammatory wound healing and fibrosis. *FASEB J* 1994;8:823-31.
14. Stoner GD, Katoh Y, Foidart JM, Trump BF, Steinert PM, Harris CC. Cultured human bronchial epithelial cells: blood group antigens, keratin, collagens, and fibronectin. *In Vitro Cell Dev Biol* 1981;17:877-87.
15. Paulsson M. Basement membrane proteins: structure, assembly, and cellular interactions. *Crit Rev Biochem Mol Biol* 1992;27:93-127.
16. Dunnill MS, Massarella GR, Anderson GA. A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmaticus, in chronic bronchitis, and in emphysema. *Thorax* 1989;24:175-9.
17. Albelda SM. Endothelial and epithelial cell adhesion molecules. *Am J Respir Cell Mol Biol* 1989;1:483-9.
18. Haahtela T, Järvinen M, Kava T, et al. Comparison of a  $\beta_2$ -agonist, terbutaline, with an inhaled corticosteroid, budesonide, in newly detected asthma. *N Engl J Med* 1991;325:388-92.
19. Haahtela T, Järvinen M, Kava T, et al. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. *N Engl J Med* 1994;331:700-5.
20. Laitinen A, Altraja A, Linden M, et al. Treatment with inhaled budesonide and tenascin expression in bronchial mucosa of allergic asthmatics. *Am J Crit Care Med* 1994; 149:A942.
21. 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.
22. Ekblom M, Fassler R, Tomasini-Johansson B, Nilsson K, Ekblom P. Downregulation of tenascin expression by glucocorticoids in bone marrow stromal cells and in fibroblasts. *J Cell Biol* 1993;4:1037-45.