

Corticosteroid treatment in bronchial asthma: For better or for worse?

Key words: Corticosteroids, apoptosis, bronchial asthma

An article by Dorscheid et al¹ in this issue of the *Journal* challenges current treatment of bronchial asthma by stating that corticosteroids, the most effective anti-asthmatic controller therapy presently available, fail to inhibit or reverse damage to the airway epithelium but rather might contribute to chronic airway damage in asthmatic patients through induction of programmed cell death (apoptosis) of airway epithelial cells. Dorscheid et al hypothesize that one of the pathologic findings observed in asthma, shedding of epithelial cells,² is at least partly caused by the use of corticosteroids to control the disease.

Bronchial asthma clearly is an inflammatory disease that is predominantly induced by allergen-mediated sensitization and chronic allergen exposure. In the course of asthmatic inflammation, release of chemotactic factors and cytokines leads to increased numbers of effector cells, such as eosinophils, basophils, and mast cells, accumulating in the peribronchial and perivascular regions of the airways. On further activation, these cells release proinflammatory mediators. Some of these mediators (eg, prostaglandins and leukotrienes) directly contribute to airway smooth muscle constriction and hypersecretion of mucus. Mediators derived from eosinophils, such as major basic protein and eosinophil peroxidase, are believed to directly damage epithelial cells and pneumocytes.^{3,4} On chronic airway inflammation, the persistent stimulation and damage of epithelial and subepithelial cells is associated with structural changes, including goblet cell metaplasia, thickening of the basement membrane, smooth muscle hypertrophy, and submucosal fibrosis (ie, remodeling).⁵ These changes are considered to be the structural correlates of the clinical findings of increased airway reactivity and decreased pulmonary function. Importantly, these histologic findings also occur in patients with mild bronchial asthma,⁶ making epithelial cell damage a feature of the disease. It is worthwhile noting that clusters of shed epithelial cells (so-called Creola bodies) were observed in the airways of asthmatic patients by Fraenkel in 1898,⁷ long before the use of corticosteroids in the therapy of asthma.

Abbreviations used

BAL: Bronchoalveolar lavage
TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling

Because of the aforementioned pathophysiologic findings in patients with bronchial asthma, anti-inflammatory approaches are required for disease control. Corticosteroids, usually administered by means of inhalation, are potent inhibitors of inflammatory responses and therefore are currently considered to be the standard therapeutic regimen for the treatment of persistent asthma.⁸ Corticosteroid treatment of asthmatic patients reduces the numbers of infiltrating eosinophils and lymphocytes in the airways and decreases production and release of proinflammatory mediators and cytokines. As a consequence, some of the structural abnormalities of asthmatic airways are normalized after corticosteroid treatment.^{9,10}

Whereas it is generally accepted that the suppressing effects of corticosteroids on inflammatory cells are at least partly caused by induction of apoptosis,⁸ little has been published about the direct apoptotic effects of corticosteroids on airway epithelial cells. To the contrary, corticosteroids have been shown to protect mammary gland epithelium and alveolar epithelial cells from apoptosis *in vitro*.^{11,12} However, in a previous report Dorscheid et al¹³ showed that *in vitro* culture of primary human epithelial cells or a human airway epithelial cell line (1HAEO-) with high concentrations of various corticosteroids induced apoptosis in a time- and concentration-dependent manner.

A major conceptual challenge of the previous study by Dorscheid et al¹³ is that they found that dexamethasone only induced apoptosis at concentrations of 3 $\mu\text{mol/L}$ or greater and that a concentration of 0.3 $\mu\text{mol/L}$ had no effect. It is important to point out that dexamethasone and the other steroids they used (eg, budesonide) are potent corticosteroids and nearly saturate the available glucocorticoid receptors at 0.3 $\mu\text{mol/L}$ (a concentration that did not induce apoptosis). Thus the *in vitro* effects observed by Dorscheid et al are unlikely to be mediated by the glucocorticoid receptor. We would predict that the glucocorticoid receptor antagonist RU486 would not block this response. We would also predict that nonglucocorticoid steroids, such as tetrahydrocortisone, testosterone, β -estradiol, and cholesterol, would also induce apoptosis in the concentration range used (3–30 $\mu\text{mol/L}$). It is unfortunate that such controls were not included in their previous study. Another conceptual challenge of this theory is that there is a body of literature showing that hydrocortisone is an important ingredient of most defined media used to

From ^athe Department of Pediatric Pneumology and Immunology, Charité, Humboldt-University, Berlin, and ^bthe Johns Hopkins University School of Medicine at the Johns Hopkins Asthma and Allergy Center, Baltimore. Received for publication December 11, 2002; accepted for publication December 11, 2002.

Reprint requests: Robert P. Schleimer, PhD, Johns Hopkins University School of Medicine at the Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Room 3A.62, Baltimore, MD 21224-6801.

© 2003 Mosby, Inc. All rights reserved.

0091-6749/2003 \$30.00 + 0

doi: 10.1067/mai.2003.156

promote the growth of epithelial cells¹⁴⁻¹⁶ and stimulates epithelial cell proliferation at relevant concentrations. In fact, recent studies by Carayol et al¹⁷ provide evidence that corticosteroids induce the β -catenin/Tcf-4 signaling pathway and inhibit the activity of caspase-3 in airway epithelial cells. Chanez et al (personal communication) propose that corticosteroids enhance epithelial repair by such a mechanism, a suggestion supported by substantial clinical literature (see below).

In their previous study Dorscheid et al¹³ nevertheless clearly demonstrate that concentrations of corticosteroids greater than those required to saturate the receptor can trigger apoptosis in epithelial cells by using a variety of accepted biochemical assays. These findings raised the possibility that corticosteroids might induce airway epithelial cell apoptosis in vivo, prompting them to determine whether this phenomenon occurs in mice. Dorscheid et al¹ present new data in this issue of the *Journal* showing that systemic treatment of mice for 3 to 6 days with a high dose of dexamethasone (1 mg/kg) resulted in increased numbers of epithelial cells in bronchoalveolar lavage (BAL) fluid. The authors do not state the methods used to unequivocally identify epithelial cells in BAL fluid and provide no information as to whether the samples were read by a blinded observer. In similarly treated animals, 3 to 6 days of dexamethasone had no effect on the number of cells staining for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and p85-poly(ADP-ribose) polymerase, 2 markers of apoptosis. It could be argued that this dissociates the appearance of epithelial cells in BAL fluid from the appearance of apoptotic cells in the epithelium. Longer treatment (2-4 weeks) with dexamethasone did lead to apoptosis in airway epithelial cells, which lasted up to 4 weeks after discontinuation of treatment and was detected by means of TUNEL staining and p85-poly(ADP-ribose) polymerase abundance. In a separate series of experiments, dexamethasone was administered after systemic sensitization and allergen airway challenges (daily dexamethasone starting 4 days after allergen airway challenge). As expected, dexamethasone significantly reduced the numbers of eosinophils in the BAL fluids and reduced the number of periodic acid-Schiff–positive (goblet) cells in tissues. Interestingly, the number of epithelial cells in the BAL fluid of dexamethasone-treated antigen-challenged animals was not increased compared with that of the antigen-challenged control animals, suggesting that the supposed apoptotic effect of dexamethasone was lost when antigen challenge was administered.

The results of Dorscheid et al¹ are in disagreement with a large body of clinical data in human subjects. Airway epithelium is fragmented and frequently found to be denuded in untreated asthma associated with increased numbers of epithelial cells in the airway lumen. Numerous studies have shown that treatment of asthmatic patients with corticosteroids causes a restitution or normalization of epithelial integrity (and/or reduces the numbers of epithelial cells in the sputum) of asthmatic

patients.^{9,10,18,19} Although there is one recent report that numbers of apoptotic cells (measured by caspase-3 activity) in bronchial biopsy specimens of asthmatic patients were increased in patients receiving corticosteroid treatment, this result might also be interpreted to reflect an increased disease activity in patients receiving corticosteroids.²⁰ Another recent clinical study of cell proliferation and apoptosis in bronchial biopsy specimens obtained from asthmatic patients showed extensive proliferative responses and little sign of apoptosis in epithelial cells from asthmatic patients regularly treated with high doses of corticosteroids, making induction of shedding by corticosteroids rather unlikely.²¹ Furthermore, numerous studies have demonstrated no histopathology associated with prolonged use of topical corticosteroids.²² Thus at present, there is little data directly supporting the hypothesis of Dorscheid et al¹ that corticosteroid treatment causes epithelial shedding in asthma.

It would be premature to make any alterations in the therapeutic use of glucocorticoids for asthma therapy on the basis of the findings of Dorscheid et al.¹ Concentrations of inhaled corticosteroids measured in the lungs of human subjects are orders of magnitude less than those found to be required by Dorscheid et al¹ to induce apoptosis in vitro.^{23,24} The mouse studies suffer from the same criticism; that is, excessively high quantities of corticosteroids have been used. Although it is always difficult to compare doses in mice and human subjects, 1 mg/kg dexamethasone is roughly equivalent to 350 mg of prednisone per day in a 70-kg human. The mouse is classified as a steroid-sensitive species, whereas human subjects are classified as steroid resistant on the basis of the ability of corticosteroids to induce apoptosis in peripheral blood lymphocytes.²⁵ Dorscheid et al¹ found evidence of epithelial apoptosis with this dose of corticosteroid only after daily treatment for 2 weeks, at which point the integrity of the lungs was compromised by treatment with systemic steroids. Extended high-dose systemic corticosteroids are well known to induce a syndrome of corticosteroid excess, including muscle wasting, extreme weight loss, and Cushingoid changes.²⁶

The proapoptotic effects of large doses of corticosteroids observed in vitro with epithelial cells and in vivo in a mouse model might add to our knowledge of the pharmacology of corticosteroids. Although we are not certain of the relevance of this effect to the use of corticosteroids systemically or by means of inhalation in asthma, an argument could be made for further studies. Among the drugs presently used to treat asthma, there are none that rival corticosteroids in their effectiveness in reducing exacerbations, emergency department visits, hospitalizations, or death. Physicians would be ill advised to alter their prescribing habits on the basis of results obtained in mice or in the culture dish with excessively high doses or concentrations of drugs.

Eckard Hamelmann, MD^a
Robert P. Schleimer, PhD^b

^aBerlin, Germany

^bBaltimore, MD

REFERENCES

1. Dorscheid DR, Low E, Conforti A, Shifrin S, Sperling AI, White SR. Corticosteroid-induced apoptosis in mouse airway epithelium: effect in normal airways and after allergen-induced airway inflammation. *J Allergy Clin Immunol* 2002;111:360-6.
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. Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000;105:651-63.
4. Coyle AJ, Uchida D, Ackerman SJ, Mitzner W, Irvin CG. Role of cationic proteins in the airway. Hyperresponsiveness due to airway inflammation. *Am J Respir Crit Care Med* 1994;150:63-71.
5. Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodeling in asthma: new insights. *J Allergy Clin Immunol* 2003;111:215-25.
6. 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.
7. Fraenkel A. *Z Kin Med* 1898;35:559. Cited by: Naylor B. The shedding of the mucosa of the bronchial tree in asthma. *Thorax* 1962;17:69.
8. National Institutes of Health, National Heart, Lung, and Blood Institute. Guidelines for the diagnosis and management of asthma. Update on selected topics. *J Allergy Clin Immunol* 2002;110:S141-219.
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 LA, Laitinen A, Heino M, Haahtela T. Eosinophilic airway inflammation during exacerbation of asthma and its treatment with inhaled corticosteroid. *Am Rev Respir Dis* 1991;143:423-7.
11. Feng Z, Marti A, Jehn B, Altermatt HJ, Chicaiza G, Jaggi R. Glucocorticoid and progesterone inhibit involution and programmed cell death in the mouse mammary gland. *J Cell Biol* 1995;131:1095-103.
12. Wen L-P, Madani K, Fahrni JA, Duncan SR, Rosen GD. Dexamethasone inhibits lung epithelial cell apoptosis induced by IFN- γ and Fas. *Am J Physiol* 1997;273:L921-9.
13. Dorscheid DR, Wojcik KR, Sun S, Marroquin B, White SR. Apoptosis of airway epithelial cells induced by corticosteroids. *Am J Respir Crit Care Med* 2001;164:1939-47.
14. Wu R, Nolan E, Turner C. Expression of tracheal differentiated functions in serum-free hormone-supplemented medium. *J Cell Physiol* 1985;125:167-81.
15. Chopra DP, Sullivan J, Wille JJ, Siddiqui KM. Propagation of differentiating normal human tracheobronchial epithelial cells in serum-free medium. *J Cell Physiol* 1987;130:173-81.
16. Kawada H, Shannon JM, Mason RJ. Improved maintenance of adult rat alveolar type II cell differentiation in vitro: effect of hydrocortisone and cyclic AMP. *Biochim Biophys Acta* 1988;972:152-66.
17. Carayol N, Vachier I, Campbell AM, Godard P, Bousquet J, Chané S. Dexamethasone induces β -catenin signaling pathway in human bronchial epithelial cells [abstract]. *Am J Respir Crit Care Med* 2002;165:A65.
18. van den Berge M, Kerstjens HA, Meijer RJ, de Reus DM, Koeter GH, Kauffman HF, et al. Corticosteroid-induced improvement in the PC20 of adenosine monophosphate is more closely associated with reduction in airway inflammation than improvement in the PC20 of methacholine. *Am J Respir Crit Care Med* 2001;164:1127-32.
19. Prosperini G, Rajakulasingam K, Cacciola RR, Spicuzza L, Rorke S, Holgate ST, et al. Changes in sputum counts and airway hyperresponsiveness after budesonide: monitoring anti-inflammatory response on the basis of surrogate markers of airway inflammation. *J Allergy Clin Immunol* 2002;110:855-61.
20. Benayoun L, Letuve S, Druilhe A, Boczkowski J, Dombret MC, Mechighel P, et al. Regulation of peroxisome proliferator-activated receptor gamma expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling. *Am J Respir Crit Care Med* 2001;164:1487-94.
21. Vignola AM, Chiappara G, Siena L, Bruno A, Gagliardo R, Merendino AM, et al. Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma. *J Allergy Clin Immunol* 2001;108:738-46.
22. Mygind N. Mucosal changes in the airways during long-term corticosteroid treatment. *Eur J Respir Dis* 1982;122:229-34.
23. Van den Bosch JMM, Westmann CJJ. Relationship between lung tissue and blood plasma concentrations of inhaled budesonide. *Biopharm Drug Dispos* 1993;14:455-9.
24. Esmailpour N, Högger P. Distribution of inhaled fluticasone propionate between human lung tissue and serum in vivo. *Eur Respir J* 1997;10:1496-9.
25. Claman HN. How corticosteroids work. *J Allergy Clin Immunol* 1975;55:145-51.
26. Axelrod L. Side effects of glucocorticoid therapy. In: Schleimer RP, Claman HN, Oronsky AR, editors. *Anti-inflammatory steroid action. Basic and clinical aspects*. San Diego: Academic Press; 1989. p. 377-408.

Bound volumes available to subscribers

Bound volumes of The Journal of Allergy and Clinical Immunology are available to subscribers (only) for the 2003 issues from the Publisher, at a cost of \$107.00 for domestic, and \$128.00 for international subscribers for Vol. 111 (January-June) and Vol. 112 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby, Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887; phone (800) 654-2452 or (407) 345-4000.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.