

Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma

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Background: Structural and functional characteristics of bronchial epithelial cells in corticosteroid-dependent asthma are unknown.

Objective: In bronchial biopsy specimens from 16 control, 9 untreated asthmatic, 9 inhaled corticosteroid-treated asthmatic, and 19 corticosteroid-dependent asthmatic subjects, we evaluated epithelium morphology and patterns of cell apoptosis, proliferation, and activation.

Methods: We used the terminal deoxynucleotidyl-mediated dUTP nick end labeling (TUNEL) technique to study apoptosis. Immunohistochemistry was used to evaluate the expression of molecules related to apoptosis (such as Bcl-2 and P53), cell proliferation (PCNA), and cell activation (NFκB and CD40/CD40-L).

Results: Epithelium thickness was higher in corticosteroid-dependent asthmatic and control subjects than in inhaled corticosteroid-treated and untreated asthmatic subjects ($P < .0001$ and $P < .0003$). Very few TUNEL-positive epithelial cells were found in the 4 groups. Bcl-2 expression was higher in all groups of asthmatic subjects than in controls ($P < .001$). In corticosteroid-dependent asthmatic subjects, PCNA, NFκB, and CD40-L expression was higher than in inhaled corticosteroid-treated asthmatic ($P < .001$), untreated asthmatic ($P < .001$ and $P < .04$), and control ($P < .01$) subjects. CD40 expression was greater in corticosteroid-dependent asthmatic and untreated asthmatic subjects than in inhaled corticosteroid-treated asthmatic subjects ($P < .0001$ and $P < .0006$) and controls ($P < .02$ and $P < .03$). In corticosteroid-dependent asthma, PCNA expression was correlated with the epithelium thickness ($P < .007$).

Conclusion: This study shows that in bronchial epithelial cells of corticosteroid-dependent asthma, markers of cell survival

and proliferation are coexpressed with markers of cell activation, suggesting that in this disease epithelium repair is associated with a persistent activation state of epithelial cells. (*J Allergy Clin Immunol* 2001;108:738-46.)

Key words: Asthma, apoptosis, proliferation, corticosteroids, inflammation

Corticosteroids represent the cornerstone treatment of chronic asthma, but though most asthmatic subjects respond to inhaled steroids,¹ a small percentage develop a severe form of the disease, needing continuous long-term treatment with oral corticosteroids^{2,3}; they are referred to as *corticosteroid-dependent asthmatic* individuals.

In asthma, the bronchial epithelium is abnormal; there are structural changes involving separation of columnar cells from their basal attachments⁴ as well as functional changes that include increased expression and release of proinflammatory cytokines,³ growth factors,⁵ and mediator-generating enzymes.⁶ We previously demonstrated that in corticosteroid-dependent asthma, bronchial epithelial cells express high levels of GM-CSF,³ a chemokine involved in leukocyte recruitment and activation,⁷ suggesting that epithelial cells might play an active role in the airway inflammation associated with the disease. However, in corticosteroid-dependent asthma both structural and functional characteristics of bronchial epithelial cells remain largely unknown. In particular, it is not known whether the injury-repair cycle in the epithelium of corticosteroid-dependent asthmatic individuals is different from that in the epithelium of untreated asthmatic individuals, asthmatic individuals treated by inhaled corticosteroids (ICSs), and control subjects.

To address this hypothesis, we evaluated in bronchial mucosal biopsy specimens taken from control subjects, untreated asthmatic subjects, asthmatic subjects treated with ICSs, and oral corticosteroid (OCS)-dependent asthmatic subjects 3 distinct biologic patterns that are involved in injury-repair processes: cell apoptosis, cell activation, and cell proliferation. Because the end result of a repair process is also promotion of the restoration of an injured tissue, we assessed the integrity of the epithelium as well.

We first evaluated epithelium thickness in bronchial biopsy specimens obtained from the 4 study groups. We then evaluated the apoptosis of bronchial epithelial cells as well as the expression of an antiapoptotic molecule,

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Abbreviations used

Bcl-2:	B-cell lymphoma leukemia 2
ICS:	Inhaled corticosteroid
LSAB:	Labeled streptavidin-biotin method
OCS:	Oral corticosteroid
PCNA:	Proliferating cell nuclear antigen
TUNEL:	Terminal deoxynucleotidyl-mediated dUTP nick end labeling

such as B-cell lymphoma leukemia 2 (Bcl-2),⁸ and of a pro-apoptotic molecule, such as P53.⁹ We also evaluated the expression of the proliferating cell nuclear antigen (PCNA) as well as the expression of the transcription factor NF κ B¹⁰ and the molecules CD40/CD40-L,^{11,12} which are important pathways for cell activation and repair.

MATERIAL AND METHODS

Subjects

Thirty-seven asthmatic subjects were selected according to the American Thoracic Society's criteria, as previously described.¹³ The clinical severity of asthma was assessed according to the Global Initiative for Asthma guidelines.¹ Subjects were distributed into 3 groups according to treatment.

The first group consisted of 9 subjects (age [years]: range, 22-55; median, 29; 25th-75th percentiles, 24.7-34.7) with mild persistent asthma who received intermittent inhaled short-acting β_2 -agonists as needed; these subjects were defined as untreated asthmatics. They had not previously been treated with ICSs, OCSs, nedocromil sodium, or cromoglycate for at least 1 month.

The second group included 9 subjects (age [years]: range, 25-48; median, 43; 25th-75th percentiles, 32.7-45.7) with moderate persistent asthma who were treated with inhaled budesonide (800 μ g daily).

A third group of 19 subjects (age [years]: range, 20-77; median, 44; 25th-75th percentiles, 31-56) with corticosteroid-dependent asthma was defined as previously described.³ All of these subjects had severe persistent asthma that required a daily dose of ICS (fluticasone propionate 1000 μ g or budesonide 1600 μ g) and an oral dose of prednisone (10 to 80 mg daily), long-acting β_2 -agonists (salmeterol 100 μ g daily), and inhaled short-acting β_2 -agonists as required. These subjects were all considered corticosteroid-dependent because trials conducted to wean them from systemic treatment during the previous 2 years had always failed.

Sixteen healthy subjects (age [years]: range, 22-45; median, 29.5; 25th-75th percentiles, 25-39) were used as controls. Their pulmonary function was within normal range.

Bronchial biopsy recoveries and tissue processing

Four biopsy specimens were recovered from each subject through use of fiberoptic bronchoscopy, performed in subsegmental bronchi as previously described.¹³ Specimens were fixed in 3.7% w/v formaldehyde and embedded in paraffin blocks; 4- μ m-thick sections were cut, deparaffined, and rehydrated before hematoxylin and eosin staining or immunohistochemistry was performed.

Evaluation of epithelium thickness

Slides were observed through use of a Leitz light microscope equipped with a $\times 10$ Dialux 22 objective (Leitz, Germany) and an EF Leitz Wetzlar JVC color video camera. Computed images were analyzed by Quantimet 500 MC software (Leica, Wetzlar, Ger-

many) for image analysis. Epithelium thickness was evaluated by a measurement of area over a minimum length of 100 μ m from the internal side of the epithelium (the ratio of area to length gives the thickness¹⁴). Results were expressed in terms of epithelium thickness (in μ m). The accuracy of the method used to measure the thickness of the epithelium was assessed by calculating coefficients of variation of 5 measures of area/length from the 4 biopsies. The coefficient of variation was <4%.

Detection of terminal deoxynucleotidyl-mediated dUTP nick end labeling (TUNEL) immunoreactivity in bronchial epithelial cells

TUNEL (Oncor, Inc, Gaithersburg, Md) was used to detect DNA strand breaks in cells undergoing apoptosis,¹⁵ as previously described in detail.⁸

Immunoreactivity of Bcl-2, P53, PCNA, CD40, CD40-L, and NF κ B

Immunoreactivity was studied on sections incubated for 45 minutes at room temperature with the following antibodies: an mAb against Bcl-2 (Dako, Glostrup, Denmark) at a dilution of 1:7; an mAb against wild-type P53 at a dilution of 1:3 (Dako); an mAb against PCNA (Dako) at a dilution of 1:10; and polyclonal antibodies against CD40 and CD40-L (Santa Cruz Biotechnology, Santa Cruz, Calif), used at a dilution of 1:30. NF κ B immunoreactivity was evaluated through use of a rabbit polyclonal anti-p65 antibody (Santa Cruz Biotechnology)¹⁶ at a dilution of 1:100. This antibody recognizes an epitope corresponding to amino acids mapping within the N-terminal domain of p65. Its specificity was assessed by Western blot analysis performed on total proteins extracted from the human bronchial epithelial cell line 16-HBE. The duration and time of incubation were in accordance with the manufacturers' instructions.

Immunoreactivity of these antibodies was revealed through use of the labeled streptavidin-biotin method (LSAB; Dako) according to the manufacturers' instructions. Control slides were prepared through use of an irrelevant mouse antibody of the same isotype and at the same concentration as the specific primary mAb (Dako).

Quantification of immunoreactive cells in bronchial epithelium

TUNEL-, Bcl-2-, P53-, CD40-, CD40-L-, NF κ B-, and PCNA-positive cell immunostainings were evaluated through use of an image analysis system (Leica Q500MC). For each biopsy specimen, the entire epithelium in 2 nonserial sections was systematically assessed on the basis of red/blue/green color balance. To ensure reproducibility of analysis, the image analysis system was standardized at the beginning of each session through use of the same section of bronchial mucosa stained either with the TUNEL technique or by the LSAB method (Dako). Through use of Quantimet software, immunoreactivity was expressed as the number of positive cells per square millimeter of epithelial area.

All of the slides were coded, and the immunoreactivity of each of the markers studied was evaluated blindly by 2 independent investigators (A.M.V. and G.C.). The interinvestigator coefficient of agreement was excellent ($\kappa = 0.94$).

Statistical analysis

Nonparametric tests were used. The Kruskal-Wallis test was used to compare the 4 groups. The Mann-Whitney *U* test was used for unpaired comparisons, and the Spearman rank test was used for correlations. For multiple correlations, Bonferroni correction was used. Results are given as medians and 25th-75th percentiles, except for some demographic characteristics.

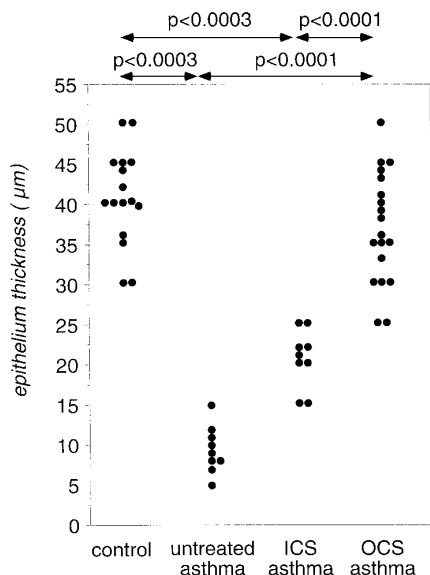


FIG 1. Evaluation of epithelium thickness in bronchial biopsy specimens of control, untreated asthmatic, ICS-treated asthmatic, and OCS-dependent asthmatic subjects. Results are expressed in μm . Statistical analysis: Mann-Whitney *U* test with Bonferroni correction.

RESULTS

Characteristics of the subjects

The demographic characteristics of the 4 study groups are presented in Table I. In OCS-dependent asthmatic subjects, airflow impairment remained high; it was similar to that in untreated and uncontrolled subjects and greater than that in ICS-treated asthmatic subjects ($P < .001$).

Thickness of bronchial epithelium

The thickness of the bronchial epithelium was similar in OCS-dependent asthmatic subjects and controls, and it was greater in these 2 groups than in ICS-treated and untreated asthmatic subjects ($P < .0001$ and $P < .0003$, respectively; Mann-Whitney *U* test; Fig 1 and Table II).

Apoptosis in epithelial cells

The numbers of apoptotic epithelial cells were not statistically different among the 4 groups of subjects studied (Figs 2 and 3 and Table II).

Immunoreactivity of antiapoptotic and proapoptotic markers

Bcl-2 immunoreactivity was significantly increased in corticosteroid-dependent asthmatic and ICS-treated asthmatic subjects in comparison with untreated asthmatic subjects ($P < .02$ and $P < .006$, respectively; Mann-Whitney *U* test) and controls ($P < .0001$; Mann-Whitney *U* test). In addition, Bcl-2 expression was higher in untreated asthmatic subjects than in controls ($P < .0001$; Mann-Whitney *U* test; Figs 2 and 4 and Table II).

The expression of P53 was found to be very low and not statistically different in the epithelia of the 4 groups of subjects studied (Fig 4 and Table II).

Expression of PCNA

PCNA immunoreactivity was significantly greater in OCS-dependent asthmatic subjects than in ICS-treated asthmatic ($P < .0007$; Mann-Whitney *U* test), untreated asthmatic ($P < .0001$; Mann-Whitney *U* test), and control ($P < .0001$; Mann-Whitney *U* test) subjects (Fig 5 and Table II). In addition, in ICS-treated asthmatic subjects, PCNA immunoreactivity was significantly greater than in untreated asthmatic subjects and controls ($P < .0001$; Mann-Whitney *U* test). Moreover, in OCS-dependent asthma, the expression of PCNA was correlated with the surface area of the epithelium layer (PCNA: $\rho = 0.63$; $P < .007$; Spearman rank correlation; Fig 6).

Expression of NF κ B and CD40/CD40-L

The expression of NF κ B was significantly higher in OCS-dependent asthmatic subjects than in ICS-treated asthmatic, untreated asthmatic, and control subjects ($P < .0001$, $P < .04$, and $P < .0001$, respectively; Mann-Whitney *U* test). In addition, NF κ B expression was higher in untreated asthmatic subjects than in ICS-treated asthmatic and control subjects ($P < .02$; Mann-Whitney *U* test; Fig 7, A and Table II). Moreover, in OCS-dependent asthma the number of epithelial cells showing a nuclear staining for NF κ B was greater than in untreated asthmatic, ICS-treated asthmatic, and control subjects ($P < .004$ and $P < .0001$, respectively; Fig 7, C).

CD40 expression was greater in OCS-dependent asthmatic and untreated asthmatic subjects than in ICS-treated asthmatic ($P < .0001$ and $P < .0006$, respectively; Mann-Whitney *U* test) and control ($P < .02$ and $P < .03$, respectively; Mann-Whitney *U* test) subjects (Fig 8 and Table II).

CD40-L expression was significantly greater in severe OCS-dependent asthmatic subjects than in ICS asthmatic, untreated asthmatic, and control subjects ($P < .0001$; Mann-Whitney *U* test). In untreated asthmatic subjects, CD40-L was slightly and significantly higher than in controls ($P < .03$; Mann-Whitney *U* test; Fig 8 and Table II).

DISCUSSION

The results of this study show that epithelial thickness is greater in OCS-dependent asthma than in untreated asthma even though the severity of the disease is increased. In ICS-treated asthma, the epithelium appears to present with a structural aspect intermediate between that seen in untreated asthma and that seen in OCS-dependent asthma. We also found that epithelial cells of all asthmatic subjects expressed higher levels of the antiapoptotic marker Bcl-2 than did epithelial cells of controls, suggesting an efficient endogenous protective mechanism against inflammatory stimuli. This finding is consistent with the low level of apoptosis found in the epithelium of mucosal biopsy specimens of asthmatic subjects. Moreover, in OCS-dependent asthmatic subjects the expression of proliferation (PCNA) and activation (NF κ B, CD40/CD40-L) markers was significantly higher than in ICS-treated and untreated asthmatic subjects.

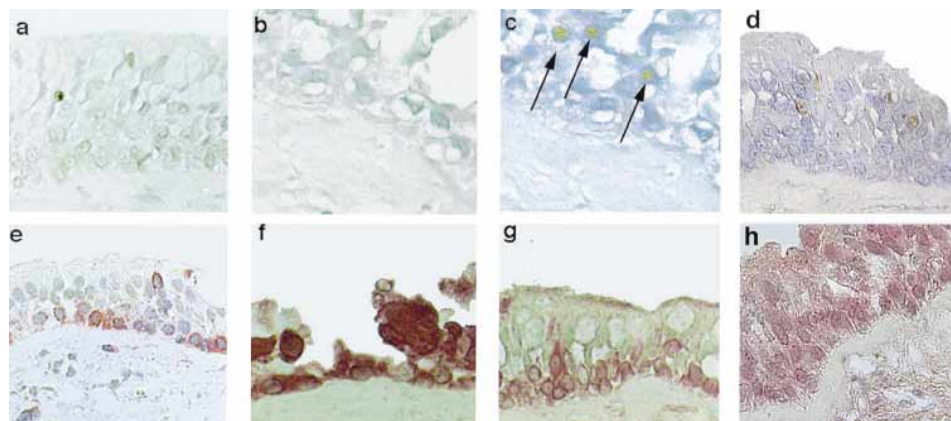


FIG 2. Evaluation of apoptosis (**a, b, c, d**) and immunoreactivity for Bcl-2 (**e, f, g, h**) in bronchial epithelial cells of a control subject (**a** and **e**), an untreated asthmatic subject (**b** and **f**), an ICS-treated asthmatic subject (**c** and **g**), and a corticosteroid-dependent asthmatic subject (**d** and **h**). Apoptotic cells are identified by dark-brown nuclear staining through use of the TUNEL technique. Few apoptotic cells are shown in panels **c** and **d** (arrows). Epithelial cells expressing Bcl-2 show a staining caused by the LSAB immunoreactivity. Final magnification: 200 $\times$.

The epithelium is an important target of airway inflammation in asthma and is frequently damaged.⁴ Epithelium damage appears to increase as the clinical severity of asthma worsens.¹⁷ Surprisingly, we found that in OCS-dependent asthma, despite the severity of the disease, the thickness of the epithelium layer was similar to that observed in controls and significantly higher than that in asthma that was untreated or treated with ICS. This apparent paradox prompted us to investigate some biological features of bronchial epithelial cells, such as cell death, survival, and proliferation. We decided to study these characteristics using bronchial biopsies for 2 major reasons: first, they offer the advantage of making it possible to study “residual” epithelial cells that have not undergone desquamation and are still attached to the basement membrane; second, such an epithelial cell presumably constitutes a sort of “stem” cell with the potential to activate the injury-repair cycle in response to inflammatory agents.¹⁸

We found that in biopsy specimens the numbers of apoptotic epithelial cells are very low; they were not statistically different in the 4 groups of subjects that we studied. Although unexpected, these results suggest that in asthma “residual” epithelial cells that have not undergone desquamation do not show a greater propensity to undergo apoptosis than epithelial cells of control subjects, a feature that might “teleologically” ensure the development of the injury-repair cycle. These data are in accord with the very low expression of P53, a proapoptotic molecule that is found in both controls and individuals with asthma.

Interestingly, in our 3 groups of asthmatic subjects the paucity of apoptotic and P53-positive cells contrasts with the high expression of Bcl-2, a molecule characterized by protective and antiapoptotic properties. Bcl-2 is important in tissue development, survival, and differentiation.^{19,20} Our results thus suggest that bronchial epithelial cells of asthmatic individuals can protect themselves

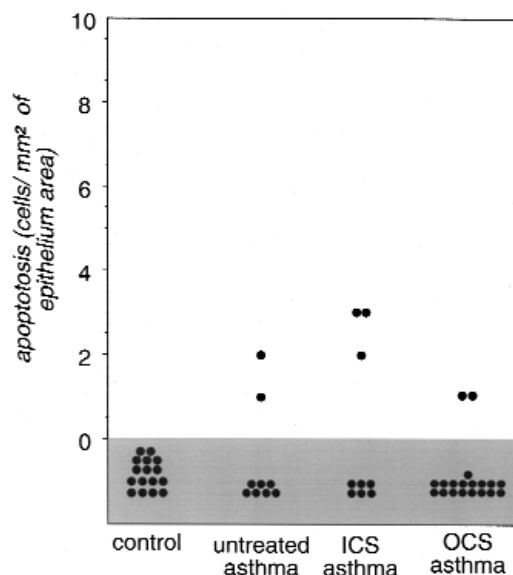


FIG 3. Numbers of apoptotic epithelial cells in control, untreated asthmatic, ICS-treated asthmatic, and OCS-dependent asthmatic subjects. Results are expressed as numbers of apoptotic cells per square millimeter of epithelium area. Statistical analysis: Mann-Whitney *U* test.

against inflammatory stimuli. In ICS-treated and OCS-dependent asthmatic subjects, the increased Bcl-2 expression throughout the epithelium layer might also account for a lack of differentiation, Bcl-2 being able to delay the terminal differentiation of epithelial cells through the prevention of apoptosis.²¹

However, such protective mechanisms might not always be sufficient to counterbalance the noxious effects of inflammatory mediators released in the airways of asthmatic individuals. This seems to occur to a greater extent in untreated asthma, in which the extensive airway

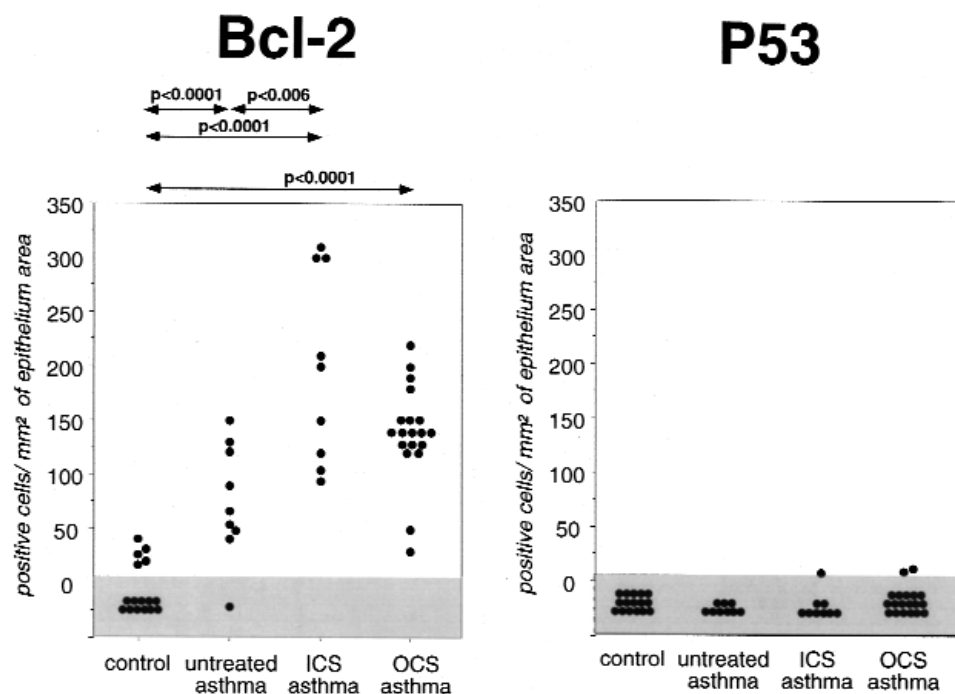


FIG 4. Numbers of bronchial epithelial cells expressing Bcl-2 and P53 in control, untreated asthmatic, ICS-treated asthmatic, and OCS-dependent asthmatic subjects are expressed as numbers of immunoreactive Bcl-2 and P53 cells per square millimeter of epithelium area. Statistical analysis: Mann-Whitney *U* test with Bonferroni correction.

TABLE I. Demographic characteristics of the subjects

Characteristic	Subject group			
	Normal	Asthmatic		
		Untreated	ICS-treated	OCS-dependent
No. of subjects	16	9	9	19
Age (y)	29.5 (25-39)	29 (24.7-34.7)	43 (32.7-45.7)	44 (31-56)
Sex (% male)	58	59	60	43
FEV ₁ (% predicted)	100 (100-105)	62 (59.5-75.7)	80.5 (79.5-82.5)	58 (48.5-63.2)
Oral prednisone dose (mg/day)	0	0	0	52 (10-60)

Results are expressed in medians; 25th-75th percentile values are shown in parentheses.

ICS, Inhaled corticosteroid; OCS, oral corticosteroid.

TABLE II. Global results of immunohistochemistry

N	Subject group				Kruskall-Wallis
	Controls	Asthmatic			
		Untreated	ICS-treated	OCS-dependent	
16	9	9	19		
Epithelium thickness (μm)	40 (38-45)	9 (8-11)	21 (19-23)	36 (31-43)	<.001
Apoptosis*	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	NS
Bcl-2*	0 (0-11)	70 (47-122)	200 (115-300)	135 (120-150)	<.001
P53*	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-0)	NS
PCNA*	0 (0-0.5)	0 (0-0)	20 (18-23)	80 (55-97)	<.001
NFκB*	0 (0-3.5)	45 (35-67)	10 (0-22)	70 (58-99)	<.001
CD40*	0 (0-0)	8 (3-14)	0 (0-0)	5 (4-9)	<.005
CD40-L*	0 (0-0)	22 (11-53)	7 (0-51)	100 (82-127)	<.001

Results are expressed in medians; 25th-75th percentile values are shown in parentheses.

ICS, Inhaled corticosteroid; OCS, oral corticosteroid; NS, not significant.

*No. of stained cells per square millimeter of bronchial epithelium.

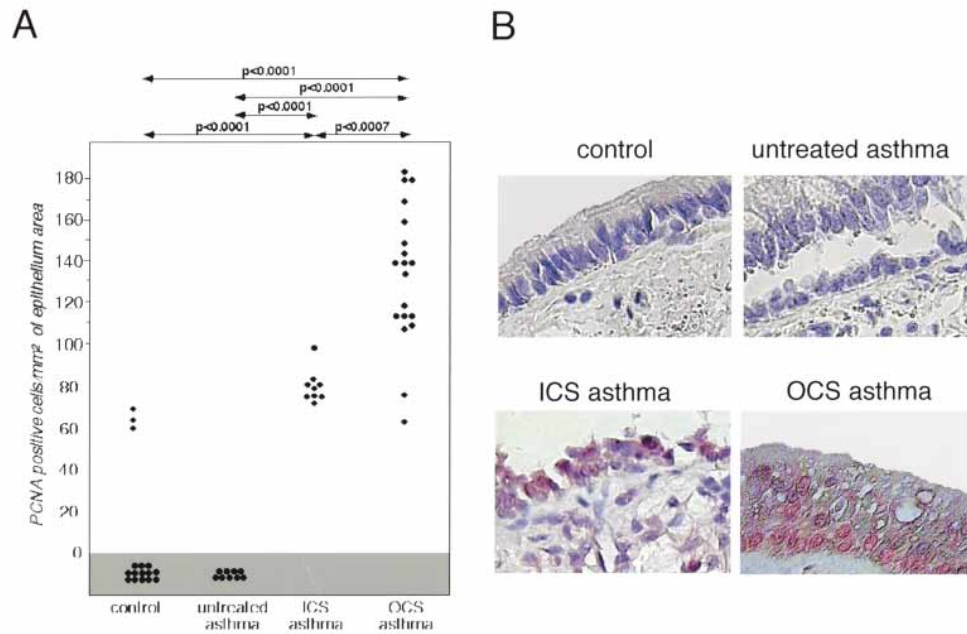


FIG 5. A, Numbers of bronchial epithelial cells expressing PCNA in control, untreated asthmatic, ICS-treated asthmatic, and OCS-dependent asthmatic subjects. Results are expressed as numbers of immunoreactive PCNA cells per square millimeter of epithelium area. **B**, Immunoreactivity for PCNA in bronchial biopsy specimens taken from a control subject, an untreated asthmatic subject, an ICS-treated asthmatic subject, and an OCS-dependent asthmatic subject. Statistical analysis: Mann-Whitney *U* test with Bonferroni correction.

epithelial damage might be a consequence of the excessive “load” of inflammatory mediators and of the inability of the epithelium to reconstitute efficiently in response to injury and/or stress. Previous studies have demonstrated a low level of PCNA expression by epithelial cells of untreated asthmatic individuals despite an extensive damage,²² revealing a potential failure in the epithelial injury-repair cycle. The present study confirms and extends this evidence, inasmuch as it shows that PCNA expression was slightly increased in ICS-treated asthmatic subjects and considerably enhanced in OCS-dependent asthmatic subjects. Although the mechanisms responsible for the increased proliferation of epithelial cells in individuals receiving corticosteroids are not completely understood, it may be speculated that the increased expression of PCNA in OCS-dependent asthma is due to a reduced transcriptional activation of the p21 molecule, a mechanism by which p53 promotes a G1 arrest of the cell cycle.²³

In addition to surviving and proliferating, bronchial epithelial cells of OCS-dependent asthma appear to be functionally activated, as shown by the increased expression of NFκB¹⁰ and of CD40/CD40-L. NFκB regulates the expression of a large number of inducible genes, such as inflammatory cytokines, growth factors, and adhesion molecules.¹⁰ An increased NFκB expression has recently been shown in bronchial epithelial cells of untreated asthmatic subjects, indicating an increased functional activation of these cells.¹⁶ In this study, we show that in OCS-dependent asthma, despite the use of high doses of corticosteroids, NFκB expression is paradoxically higher

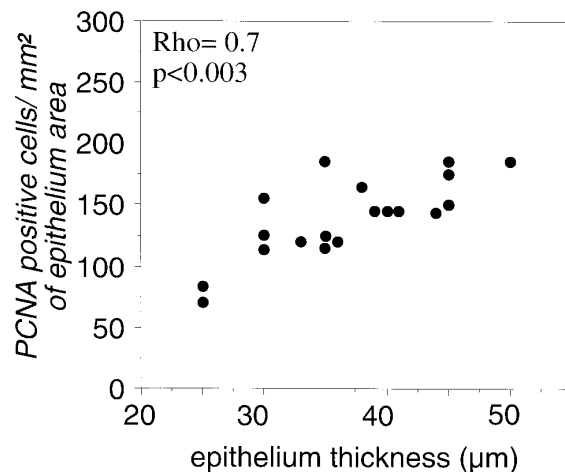


FIG 6. Correlation between the expression of PCNA and the area of the epithelium, as measured in mucosal biopsy specimens of corticosteroid-dependent asthma. Statistical analysis: Spearman rank test.

than in untreated asthma. These data support a reduced cellular responsiveness to corticosteroids but might also suggest the ability of corticosteroids to induce p65 protein expression.²⁴ We also found that expression of p65 in the nuclei of airway epithelial cells was higher in the corticosteroid-dependent asthmatic group than in the other study groups, suggesting a greater activation state of these cells and a possible reduced cellular responsiveness to glucocorticosteroids in OCS-dependent subjects. In addition,

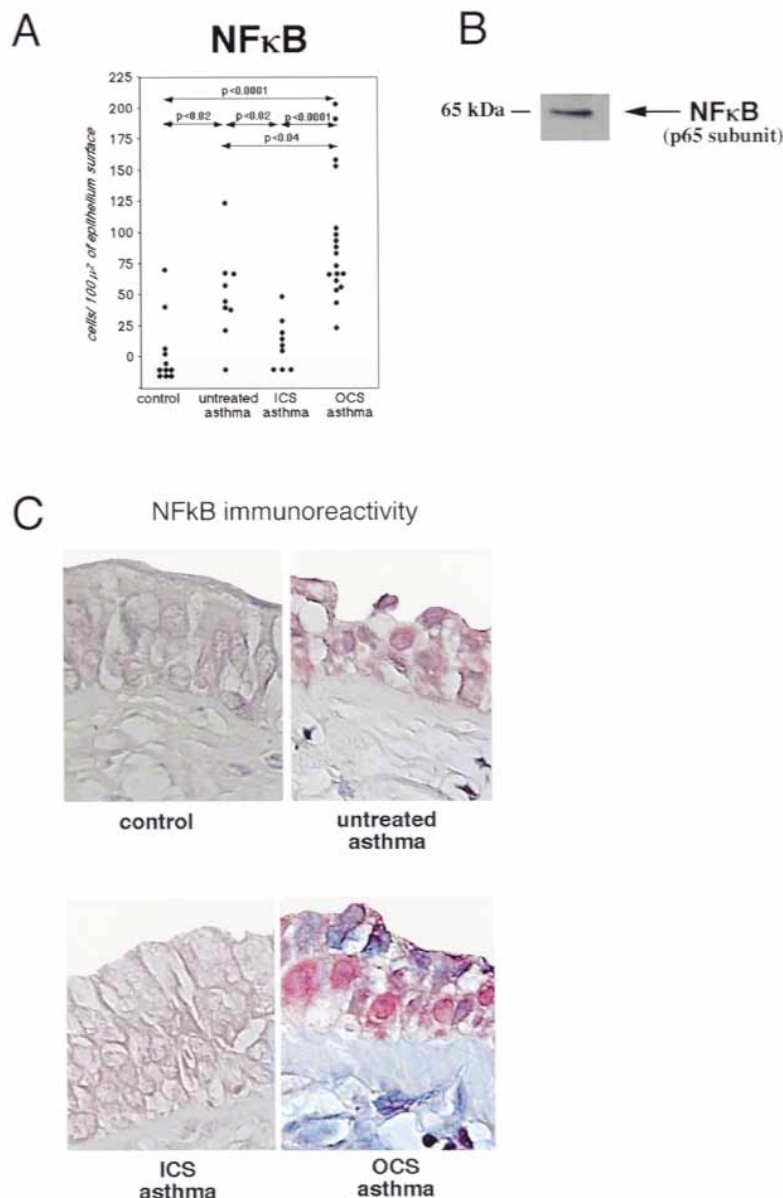


FIG 7. NF κ B immunoreactivity in bronchial biopsy specimens of control, untreated asthmatic, ICS-treated asthmatic, and OCS-dependent asthmatic subjects. **A**, Expression of NF κ B by bronchial epithelial cells in biopsy specimens from the 4 study groups. Results are expressed as numbers of NF κ B-positive cells per square millimeter of epithelium surface. **B**, Representative Western blot analysis of NF κ B (p65 subunit) protein expression performed on total proteins extracted from the human bronchial epithelial cell line 16-HBE. **C**, Immunoreactivity of bronchial epithelial cells for NF κ B in bronchial biopsy specimens taken from a control subject, an untreated asthmatic subject, an ICS-treated asthmatic subject, and a corticosteroid-dependent asthmatic subject. NF κ B expression, shown by the red staining caused by LSAB immunoreactivity, is increased in the epithelium of corticosteroid-dependent asthmatic subjects and shows a nuclear staining for NF κ B.

this evidence is consistent with previous evidence obtained by our group showing that bronchial epithelial cells of steroid-dependent asthmatic subjects express very high levels of GM-CSF,³ a powerful proinflammatory cytokine.

We also examined the expression of CD40/CD40-L molecules, because they control an important pathway for cell activation, mediate signal transduction (which might include NF κ B activation),²⁵⁻²⁷ and induce the

release of TNF- α and IL-8,¹¹ two cytokines that promote the recruitment of neutrophils in OCS-dependent asthma.² In addition, CD40 expression by basal epithelium can influence commitment to their differentiation.^{28,29} In this study, we show an increased expression of CD40/CD40-L in bronchial epithelial cells of OCS-dependent asthmatic subjects. It might be proposed that in these individuals the interaction between CD40 and its

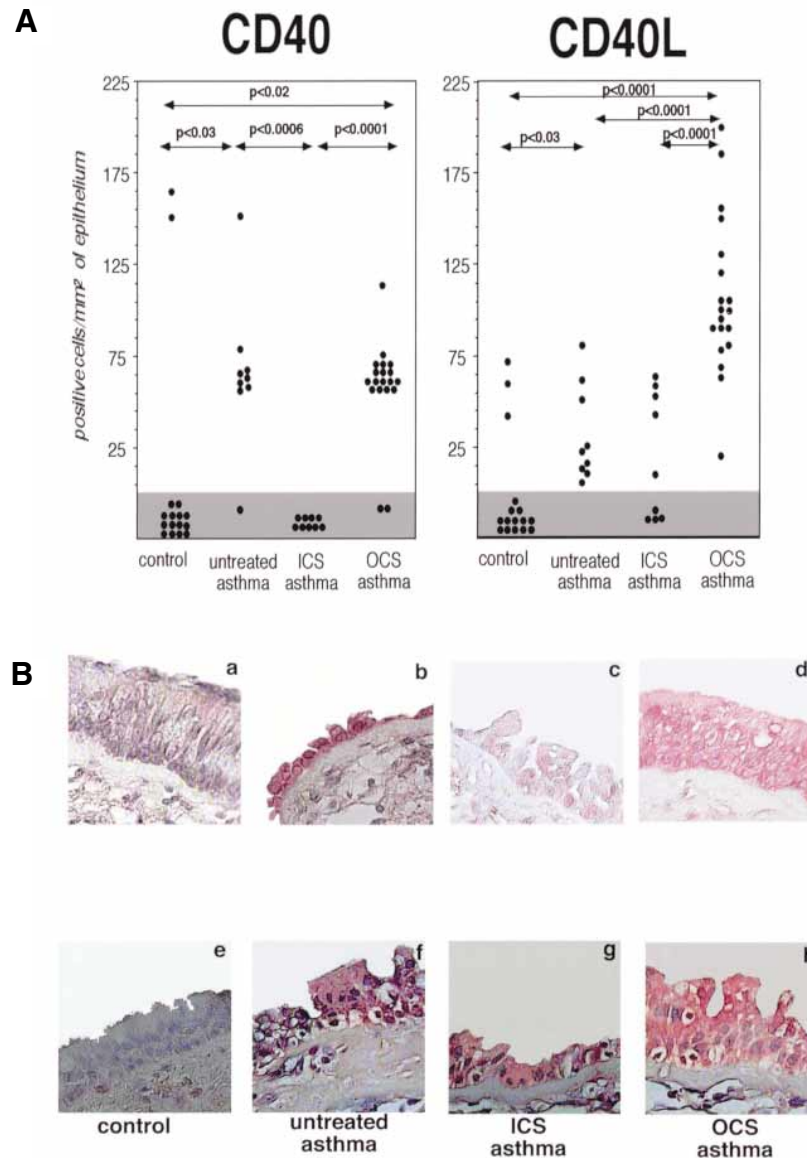


FIG 8. A, Expression of CD40 and CD40-L in bronchial biopsy specimens of control, untreated, and corticosteroid-dependent asthmatic subjects. Immunoreactivity for CD40 and CD40-L is expressed as the number of positive cells per square millimeter of epithelium area. Statistical analysis: Mann-Whitney *U* test with Bonferroni correction. **B**, Microphotographs show the immunoreactivity for CD40 (upper panels) and CD40-L (lower panels) in bronchial biopsy specimens from a control subject (a and e), an untreated subject (b and f), an ICS-treated asthmatic subject (c and g), and a corticosteroid-dependent asthmatic subject (d and h). Final magnification: 100 $\times$.

ligand is functional and plays a role in the control of epithelial cell activation and differentiation. Both of these effects might have important pathophysiologic consequences in OCS-dependent asthma. CD40/CD40-L interaction might be responsible for a persistent activation of bronchial epithelial cells,³ contributing to the chronic inflammation of the airways. In addition, CD40/CD40-L interaction between adjacent epithelial cells might indeed represent a further mechanism capable of enhancing the differentiation of these cells.¹²

In conclusion, this investigation provides evidence that

in asthma, epithelial cells can express antiapoptotic molecules such as Bcl-2. However, though in untreated asthmatic subjects such a response does not appear to be associated with an increased cell proliferation, in ICS-treated asthmatic subjects and, to a greater extent, in OCS-dependent asthmatic subjects epithelial cells show an enhanced proliferative response. Finally, despite the use of corticosteroids, in this latter group of subjects epithelial cells exhibit an enhanced state of activation that might play an important role in the development and persistence of airway inflammation associated with OCS-dependent asthma.

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