Mechanisms of glucocorticoid insensitivity in asthma

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Chapter 3

Short Communication: Smoking and inhaled corticosteroid use are independently associated with higher histone deacetylase-2 expression in bronchial epithelial cells in asthma

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Corticosteroids, the most effective therapy available for asthma, suppress inflammatory genes by inhibiting histone acetyltransferase (HAT) and in particular by recruiting histone deacetylase-2 (HDAC-2) to the nuclear factor-κB-activated inflammatory gene complex[1]. The epithelium is a major site of inflammatory gene expression and a main localization site for HDAC-2 expression within the airway wall, and therefore target for (inhaled) corticosteroid action[2]. Examination of complete bronchial biopsies from non-smoking mild/moderate persistent asthmatics treated with budesonide revealed higher HDAC-2 levels compared to non-smoking mild intermittent asthmatics untreated with budesonide[2].

Cigarette smoke has been shown to down-regulate expression and activity of HDAC-2 in bronchial biopsies and alveolar macrophages from young healthy smokers.[3] Reduced HDAC-2 expression was suggested as one of the underlying mechanisms for reduced corticosteroid responsiveness in COPD, severe asthma and smoking asthma.[1] However, HDAC-2 expression data in bronchial epithelial cells in severe asthma did not support this hypothesis[4;5]. Moreover, a recent study unexpectedly demonstrated that bronchial epithelial cells from COPD patients and healthy smokers have *higher* HDAC-2 expression than healthy non-smokers[6]. Until now, HDAC-2 expression of bronchial epithelial cells has not been investigated in smoking asthmatics. Because epithelial cells constitute the first barrier affected by smoking, we compared epithelial HDAC-2 expression in smoking and non-smoking asthmatics, taking into account inhaled corticosteroids (ICS) use.

ICS= inhaled corticosteroid, Atopy is based on a positive phadiatop, FEV_1= forced expiratory volume in 1 s (FEV_1 was measured after inhalation of 800 µg Albuterol), Reversibility FEV_1= change in FEV_1, expressed as increase in percentage predicted normal value after 400 µg of Albuterol, PC_{20} AMP (mg/ml)= provocative concentration of adenosine 5'-monophosphate causing a 20% fall in $_{FEV1.}$ *= non-smoking ICS use vs. smoking ICS use $p<0.05$, #= smoking non-ICS use vs. non-smoking non-ICS use $p<0.05$, $= non-smoking ICS use vs. non-smoking non-ICS use $p<0.05$, †= smoking non-ICS use vs. non-smoking ICS use $p<0.05$, ‡= smoking non-ICS use vs. smoking ICS use $p<0.05$, ∞= smoking ICS use vs. non-smoking non-ICS use $p<0.05$.

Endobronchial biopsies from 96 non-smoking asthmatics and 27 current smoking asthmatics (Table 1) were immunostained for HDAC-2. The percentage of HDAC-2 nucleus positive cells was determined in the intact epithelium (Figure 1) and compared between asthmatics with and without ICS treatment.

Table 1. Characteristics of asthma patients

Characteristics	Non-smoking non-ICS use (50 cases)	Smoking non-ICS use (19 cases)	Non-smoking ICS use (46 cases)	Smoking ICS use (8 cases)
Female sex (%)	26 (52%)	5 (35.7%)	24 (52.2%)	4 (50%)
Age (yr)	47 (25-70)	52 (19-64)	50.5 (19-71)*	36.5 (24-64)
Packyears (yr)	0 (0-44.6)	25.6 (1.4-44)#,†,‡	0.2 (0-63.8)*,§	5.6 (0.4-33.6)¤
Cigarettes/day (n)	0 (0-18)	15 (3-23)	0 (0-0)	10 (3-14)
ICS dose (µg/day) beclomethasone equivalent	--	--	800 (28-2000)	650 (200-1000)
Atopy (%)	33 (66%)	13 (68.4%)	35 (76.1%)	6 (75%)
FEV_1 (% pred)	104 (77.9-127.8)	94.6 (59.8-134.5)	96.3 (42.5-135.5)§	101.4 (81.3-108.6)
FEV_1/VC (%)	78.2 (56.4-97.7)	71.9 (47.6-93.6)	72.5 (39.4-96.7)§	75.9 (54.9-84.4)
Reversibility (% pred)	6.2 (-1.4-28.7)	9.2 (-2.2-17.8)	7.5 (-0.8-38.4)	10.5 (4.6-25.6)
Log PC_{20} AMP	2.8 (-1.7-2.8)	2.3 (-1.7-2.8)	2.3 (-2.6-2.8)	0.9 (0.6-1.5)*,‡,¤

Values are medians (ranges) or numbers (proportions).

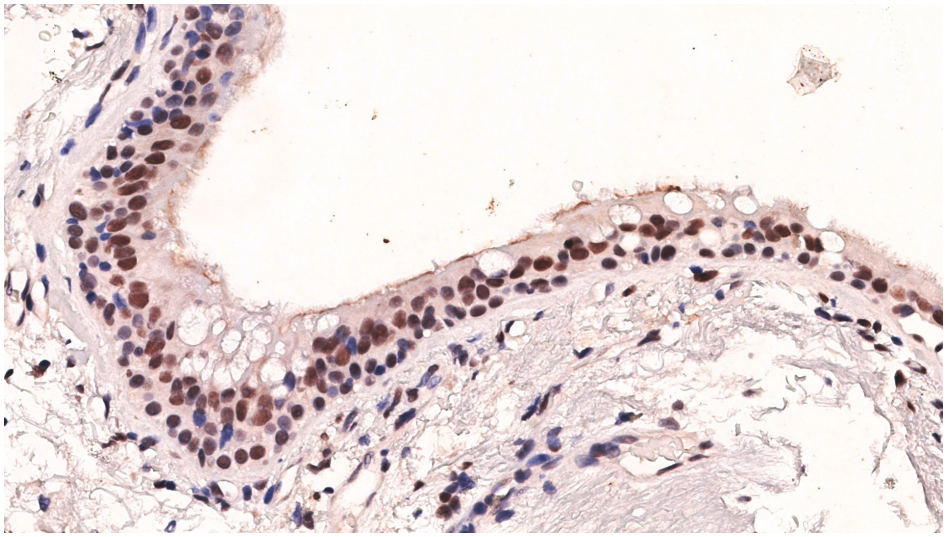

Figure 1. Expression of HDAC-2 in a representative bronchial biopsy from a non-smoking asthmatic on ICS use.

Our results show that current smoking is associated with higher HDAC-2 expression in the epithelium in asthmatics not treated with ICS (Figure 2). ICS-use is associated with higher HDAC-2 expression in non-smoking asthmatics, but we did not observe this in currently smoking asthmatics. Linear regression analysis confirmed that smoking and ICS-use contribute independently to HDAC-2 expression (B: 14.47, 95% CI: 8.21–20.73 and B: 10.86, 95% CI: 6.12–15.61 respectively). Smoking interacted *negatively* with ICS-use (B: -19.62, 95% CI: -30.50– -8.74).

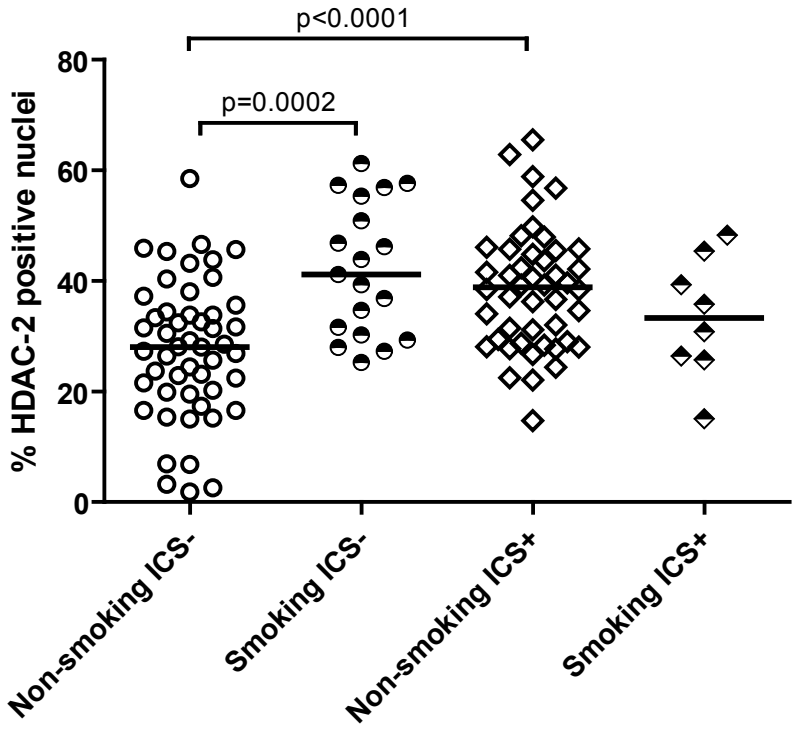

Figure 2. Percentage of HDAC-2 nucleus positive cells in intact epithelium.

Our study for the first time demonstrated that HDAC-2 expression is higher in epithelial cells from smoking asthmatics. This is compatible with recent findings in epithelial cells from healthy smokers and COPD patients[6], but incompatible with findings in alveolar macrophages from healthy smokers[3] and COPD patients[7]. Together, these observations suggest that effects of smoking on HDAC-2 expression may vary between compartments. It is intriguing that smoking might lead to higher HDAC-2 expression in epithelial cells, suggesting an anti-inflammatory effect in that compartment.

Additionally, HDAC-2 expression was higher in bronchial epithelial cells from non-smoking asthmatics using ICS, in line with previous findings.[2] We did not observe this beneficial effect of ICS on HDAC-2 expression in smoking asthmatics, reflecting a possible mechanism for smoking-induced corticosteroid unresponsiveness. However, we cannot draw a definitive conclusion because we did not investigate the balance between HAT and HDAC-2, nor their activity. Additionally, we performed an observational study, not randomizing for corticosteroids or smoking. Therefore, further studies on smoking-induced corticosteroid unresponsiveness in asthma need to confirm our preliminary results.

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