Cigarette smoking is associated with lower body mass index, and cessation of smoking is associated with weight gain.\textsuperscript{1,2} Cross-sectional studies show that smokers weigh less than age-matched nonsmokers,\textsuperscript{1,3–7} and longitudinal data show that most smokers gain weight after smoking cessation.\textsuperscript{2,8–16} The mechanisms underlying the lower weight of smokers are undoubtedly complex, and a variety of studies have linked it to decreased food intake, increased metabolic rate, increased physical activity, and the metabolic effects of nicotine.\textsuperscript{1,17–27}

In the present study we propose an additional mechanism that may contribute to the smoking-associated weight loss, based on the hypothesis that smoking may induce the up-regulation in the respiratory epithelium of genes that code for proteins associated with weight loss.\textsuperscript{28,29} To evaluate this concept, we assessed our microarray data of airway epithelial gene expression in healthy smokers and healthy nonsmokers for genes that have been reported to be associated with mediating weight loss.\textsuperscript{28,29} Strikingly, the data showed that smoking...
markedly up-regulated the airway epithelial expression of \(\alpha_2\)-zinc-glycoprotein 1 (AZGP1), a gene associated with the up-regulation of uncoupling proteins that have been implicated in regulating energy balance and body weight.\(^{28-32}\)

AZGP1, a 38- to 41-kd peptide normally found in body fluids, functions as a lipid mobilizing factor.\(^{29,33-36}\) AZGP1 is found in the urine of cancer patients with cachexia,\(^{36}\) is overexpressed in carcinomas associated with fat loss,\(^{34,37}\) and mice treated with AZGP1 have a significant decrease in body fat, without a change in food or water intake.\(^{35}\) AZGP1 is known to be normally expressed in the secretory epithelia of the liver, breast, GI tract, sweat glands, and of interest to the present study, the lung.\(^{38,39}\)

The name AZGP1 is based on the knowledge that it precipitates with zinc salts and has electrophoretic mobility similar to that of \(\alpha_2\)-globulins.\(^{32}\) The mechanisms of AZGP1 are not fully understood, but it is believed to regulate lipid degradation through activation of guanosine triphosphate-dependent adenylate cyclase activity mediated through the \(\beta_3\)-adrenoreceptor.\(^{35,40}\)

Based on the knowledge that increased body fat and weight gain are observed following smoking cessation, lower body mass index is associated with cigarette smoking, adipose tissue metabolism appears to be altered in smokers, and our preliminary observations, we further investigated whether cigarette smokers up-regulated the expression of AZGP1 in the human airway epithelium. The analysis included assessment of AZGP1 gene expression of large airway epithelium obtained by fiberoptic bronchoscopy and brushings from healthy nonsmokers and healthy smokers using microarrays with TaqMan polymerase chain reaction (PCR) confirmation. The data demonstrate that the expression of AZGP1 is significantly up-regulated in healthy smokers. Western blot analysis of large airway biopsy specimens confirmed the up-regulation of AZGP1 in smokers compared to nonsmokers at the protein level. Interestingly, anti-AZGP1 immunofluorescence assessment of large airway bronchial biopsy specimens and brushed large airway epithelium specimens showed that, in addition to being up-regulated in secretory cells of smokers, AZGP1 is expressed in neuroendocrine cells of smokers. In the context that AZGP1 is linked to weight loss, the up-regulation of AZGP1 in the airway epithelium of healthy smokers may represent a pathway contributing to the weight loss associated with smoking.

**Materials and Methods**

**Study Population**

Healthy nonsmokers and healthy current cigarette smokers were evaluated at the Weill Cornell NIH General Clinical Research Center and Department of Genetic Medicine Clinical Research Facility under protocols approved by the Weill Cornell Medical College Institutional Review Board. Written informed consent was obtained from each individual before enrollment in the study. Nonsmokers and smokers were determined to be phenotypically healthy on the basis of clinical history, physical examination, routine blood screening tests, urinalysis, chest radiograph, ECG, and pulmonary function testing. No individual in either study group had any evidence of a malignancy. Current smoking status was confirmed by history, venous carboxyhemoglobin levels, and urinalysis for nicotine levels and its derivative cotinine. Individuals who met the inclusion criteria underwent fiberoptic bronchoscopy with brushing and/or endobronchial biopsy.

**Collection of Airway Epithelial Cells**

Epithelial cells from the large airways were collected using flexible bronchoscopy. Smokers were asked not to smoke the evening prior to the procedure. After achieving mild sedation and anesthesia of vocal cords, a flexible bronchoscope (EB-1530T3; Pentax; Tokyo, Japan) was advanced to the desired bronchus. Large airway epithelial samples were collected by gentle brushing of the third- to fourth-order bronchi. The epithelial cells were subsequently collected in 5 mL of 4°C LHCS medium ( Gibco; Grand Island, NY). An aliquot of this was used for cytology and differential cell count, and the remainder was processed immediately for RNA extraction. Total cell counts were obtained using a hemocytometer while differential cell counts were determined on sedimented cells prepared by centrifugation (Cytopsin 11; Shandon Instruments; Pittsburgh, PA) and stained (Diff-Quik; Baxter Healthcare; Miami, FL).

**RNA Extraction and Microarray Processing**

Analyses were done using the following three different microarrays from Affymetrix (Santa Clara, CA): HuGeneFL (7,000 probe sets); HG-U133A (22,000 probe sets); and HG-U133 Plus 2.0 (54,000 probe sets). The protocols used were as described by the manufacturer. Total RNA was extracted from epithelial cells (TRizol; Invitrogen; Carlsbad, CA) with further cleanup (RNeasy; Qiagen; Valencia, CA). This process yielded 2 to 4 µg RNA per 10^6 cells. Samples were processed as previously de-
scribed using the kits and methods purchased from Affymetrix. Hybridizations to test chips and to the microarrays were done according to the protocols of the manufacturer (Affymetrix), and microarrays were processed using a fluids station (Affymetrix) and scanned (for the HuGeneFL microarray, GeneArray 2500; Affymetrix; for the HG-U133A and HG-U133 Plus 2.0 microarrays, GeneChip Scanner 3000 7G; Affymetrix). To maintain quality, only samples hybridized to test chips with a glyceraldehyde phosphate dehydrogenase of 3' to 5' ratio of < 3 were deemed satisfactory.

Microarray Data Analysis

Captured images were analyzed (Microarray Suite, version 5.0 algorithm; Affymetrix). These data were normalized using appropriate software (GeneSpring, version 7.2; Agilent Technologies; Palo Alto, CA) as follows: (1) per array, by dividing raw data by the fiftieth percentile of all measurements; and (2) per gene, by dividing the raw data by the median expression level for each gene across all arrays in a data set.

TaqMan Reverse Transcription-PCR Confirmation of Microarray Expression Levels

TaqMan real-time reverse transcription-PCR (RT-PCR) was done on RNA samples from the large airways of 17 healthy nonsmokers and 15 healthy smokers that had also been assessed (HG-U133 Plus 2.0 microarray; Affymetrix). Complementary DNA was synthesized from 2 µg of RNA in a 100-µL reaction volume (TaqMan Reverse Transcription Reaction kit; Applied Biosystems; Foster City, CA), with random hexamers as primers. Dilutions of 1:10 and 1:100 were made from each sample, and triplicate wells were run for each dilution. TaqMan PCR reactions were carried out using premade gene expression assays for the AZGP1 gene (Applied Biosystems), and 2 µL of complementary DNA were used in each 25-µL reaction volume. The endogenous control was 18S ribosomal RNA, and relative expression levels were determined using the ΔΔCt method (Applied Biosystems) with the average value for the nonsmokers as the calibrator. The PCR reactions were run in a sequence detector (Sequence Detection System 7500; Applied Biosystems).

Localization of AZGP1 in the Large Airway Epithelium

To assess which airway epithelial cells express AZGP1, bronchial biopsy specimens were obtained from the large airways of healthy nonsmokers and healthy smokers using conventional methods. Immunohistochemistry was subsequently done on paraffin-embedded endobronchial biopsy specimens. Sections were deparaffinized and rehydrated through a series of xylenes and alcohol. To enhance staining, an antigen recovery step was carried out by microwave treatment at 100°C, 15 min in citrate buffer solution (Labvision; Fremont, CA) followed by cooling at 23°C for 20 min. Endogenous peroxidase activity was quenched using 0.3% H2O2, and normal goat serum was used to reduce background staining. Samples were incubated with the primary antibody rabbit polyclonal anti-AZGP1 (1/5,000 dilution; Biovendor; Candler, NC) at 4°C overnight. Rabbit IgG (Jackson ImmunoResearch) as the secondary antibody for the AZGP1 (Jackson ImmunoResearch) was used as a secondary antibody for AZGP1, and goat antihuman IgG (Jackson ImmunoResearch) was used as a secondary antibody for chromogranin A and mucin 5AC. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, 1/200 dilution; Molecular Probes). A total of nine slides were used for each airway epithelial cell sample, as follows: (1) AZGP1 alone; (2) rabbit IgG control for AZGP1; (3) chromogranin A alone; (4) mucin 5AC alone; (5) mouse IgG control for chromogranin and mucin 5AC; (6) AZGP1 and chromogranin A colocalization; (7) rabbit IgG control for AZGP1 and mouse IgG control for chromogranin A; (8) AZGP1 and mucin 5AC colocalization; and (9) rabbit IgG control for AZGP1 and mouse IgG control for mucin 5AC. Images were captured using an Olympus IX 70 fluorescence microscope with 60-fold magnification. Images were analyzed using appropriate software (MetaMorph; Universal Imaging Corporation; Downingtown, PA). Pseudocolor images were formed by encoding Cy5 fluorescence in the green channel and Cy3 fluorescence in the red channel.

Quantitation of AZGP1-Positive Cells

To compare the frequency of AZGP1-positive cells between healthy nonsmokers and healthy smokers, cytospin preparations of large airway epithelial cells from 5 nonsmokers and 6 smokers were stained with rabbit antihuman AZGP1 or rabbit IgG as control. Goat antirabbit Cy3 was used as a secondary antibody. Nuclei were counterstained with DAPI. The percentage of AZGP1-positive cells was calculated as the number of AZGP1-positive cells per field/total number of nuclei per field in 10 random fields for each subject of the nonsmoking and smoking groups compared by t test.

Western Analysis

Western analysis was used to quantitatively assess AZGP1 protein expression in large airway brushing samples from healthy nonsmokers and healthy smokers. Brushed large airway epithelial cells were obtained as described. Initially, the cells were centrifuged at 600g, 5 min, 4°C. The whole cells were lysed with red cell lysis buffer (Cell Lytic Mammalian Tissue Lysis/Extraction reagent; Sigma-Aldrich) followed by cell lysis buffer (ACK lysis buffer; Invitrogen), and protease inhibitor (Sigma-Aldrich) was added to the sample. The sample was centrifuged at 10,000g and the protein-containing supernatant collected. The protein concentrations were assessed using a bicinchoninic acid protein concentration kit (Pierce; Rockford, IL). An equal concentration of protein (10 µg) mixed with sodium dodecyl sulfate (SDS Sample Loading Buffer; Invitrogen) and a reducing agent was

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loaded on Tris-glycine gels (Invitrogen). Protein electrophoresis was carried out at 100 V, for 2 h, and at 23°C. Sample proteins were transferred (at 25 V, for 1 h, and at 4°C) to a 0.45-μm-thick polyvinylidene fluoride membrane (Invitrogen) using a power source (Power Pack 300; Bio-Rad; Hercules, CA) and Tris-glycine transfer buffer (Bio-Rad). After transfer, the membranes were blocked with 5% milk in phosphate-buffered saline for 1 h, 23°C. The membranes were incubated with primary rabbit polyclonal anti-AZGP1 antibody (Biovendor) at 1:1,000 dilution, for 2 h, at 4°C. Recombinant AZGP1 protein (Biovendor) was used as a positive control. Detection was performed using horseradish peroxidase-conjugated antirabbit antibody (1:2,000 dilution; Santa Cruz Biotechnology; Santa Cruz, CA) and a chemiluminescent reagent system (ECL; GE Healthcare; Pittsburgh, PA) using enhanced chemiluminescence (Hyperfilm; GE Healthcare). To assess the Western analyses quantitatively, the film was digitally imaged, maintaining exposure within the linear range of detection. The contrast was inverted, the pixel intensity of each band determined, and the background pixel intensity for a negative area of the film of identical size subtracted using image analysis software (MetaMorph; Universal Imaging). The membrane was subsequently stripped and reincubated with horseradish peroxidase-conjugated anti-β-actin antibody (Santa Cruz Biotechnology) as a control for equal protein concentration.

**Statistical Analysis**

Average expression values for AZGP1 in large airway samples were calculated from normalized expression levels for healthy nonsmokers and healthy smokers, and p values for all comparisons were calculated using the two-sample unequal variance Welch t test without correction for multiple testing.

**Web Deposition of Data**

All data have been deposited in the Gene Expression Omnibus (GEO) site (http://www.ncbi.nlm.nih.gov/geo), curated by the National Center for Biinformatics. The accession number is GSE10135.

**Role of the Funding Source**

The funding source of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report or the decision to submit this report for publication.

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**Table 1—Study Population and Large Airway Epithelial Samples**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HuGeneFL Microarray</th>
<th>HG-U133A Microarray</th>
<th>HG-U133 Plus 2.0 Microarray</th>
</tr>
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<tr>
<td></td>
<td>Healthy Nonsmokers</td>
<td>Healthy Smokers</td>
<td>Healthy Nonsmokers</td>
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<tr>
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<td>(n = 13)</td>
<td>(n = 5)</td>
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<td>Age, yr</td>
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<td>38 ± 2</td>
<td>34 ± 2</td>
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<tr>
<td>Race, No.</td>
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<tr>
<td>Asian</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Smoking history, pack-yr</td>
<td>0</td>
<td>21 ± 4</td>
<td>0</td>
</tr>
<tr>
<td>Urine nicotine,† ng/mL</td>
<td>8 ± 7</td>
<td>3,493 ± 308</td>
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<tr>
<td>Urine cotinine, ng/mL</td>
<td>Negative</td>
<td>1,118 ± 204</td>
<td>34 ± 7</td>
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<td>Venous CO-Hb†</td>
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<td>4.0 ± 0.5</td>
<td>0.5 ± 0.05</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>Undifferentiated</td>
<td>21 ± 6</td>
<td>24 ± 3</td>
<td>21 ± 4</td>
</tr>
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</table>

*Values are given as the mean ± SD. Diff = diffusing capacity of the lung for carbon monoxide; TLC = total lung capacity; Venous CO-Hb = venous carboxyhemoglobin.
†Venous CO-Hb is a secondary marker of current smoking; nonsmokers; < 1.5%.
‡Combined urine nicotine and cotinine level of < 50 ng/mL is considered negative.
Results

Study Population and Biological Samples

A total of 92 individuals, 37 healthy nonsmokers and 55 healthy smokers, were included in the microarray assessment of expression profiles of the large airway epithelium. These included 9 healthy nonsmokers and 13 healthy smokers from the HuGeneFL data set, 5 healthy nonsmokers and 6 healthy smokers from the HG-U133A data set, and 23 healthy nonsmokers and 36 healthy smokers from the HG-U133 Plus 2.0 data set (Table 1, Fig 1, online supplemental Fig 1). All individuals had normal general physical examination findings and no significant findings in the medical history. There were no differences between groups with regard to gender, race, or age (p > 0.05). All individuals were HIV negative with blood and urine parameters within normal ranges (p > 0.05 for all comparisons). The mean (± SD) body mass indices of the 37 healthy nonsmokers and 55 healthy smokers were 25.3 ± 3.6 and 27.7 ± 6.1 kg/m², respectively, and the values were not significantly different (p > 0.05). Healthy smokers had a mean history of smoking of 27 ± 2 pack-years, and measurement of venous blood carboxyhemoglobin levels and urine nicotine and cotinine levels confirmed the smoking status of these individuals. Pulmonary function testing revealed normal lung function in healthy nonsmokers and healthy smokers (Table 1). The number of airway epithelial cells recovered ranged from 6.9 to 9.4 x 10⁶ cells (Table 1). In all cases, > 96% of the cells recovered were epithelial cells. The various categories of airway epithelial cells were as expected for large airways.⁴⁴

Expression of AZGP1 in the Large Airway Epithelium

Using the criteria of the Detection Call of Present (P call; Affymetrix) in ≥ 50% of patients, AZGP1 was significantly up-regulated in the large airway epithelium of healthy smokers compared to healthy nonsmokers in every data set (Fig 1, online supplemental Fig 1). Assessment of GEO deposited microarray data from the independent data set of Spira et al⁴⁵ confirmed the significant expression level of AZGP1 in the large airway epithelium of smokers (Fig 2).

To confirm the results obtained from microarray studies, TaqMan RT-PCR was carried out on RNA samples from the large airways of 17 healthy nonsmokers and 36 healthy smokers.
smokers and 15 healthy smokers (Fig 3). The TaqMan data confirmed the up-regulation of AZGP1 messenger RNA expression in healthy smokers compared to that in healthy nonsmokers (1.9 ± 0.34-fold increase; p < 0.05).

Western analysis was carried out on large airway samples from a total of 10 healthy nonsmokers and 10 healthy smokers to quantitatively assess AZGP1 expression. Overall, healthy smokers had increased AZGP1 expression when compared to nonsmokers (Fig 4, top, A). Analysis of the digitally imaged film (MetaMorph image analysis software; Universal Imaging Corporation) revealed significantly increased AZGP1 protein expression in healthy smokers compared to healthy nonsmokers (Fig 4, bottom, B; p < 0.02).

Immunohistochemistry of large airway epithelial biopsy specimens obtained from healthy nonsmokers and healthy smokers was used to assess the cell-specific expression of AZGP1 (Fig 5). Positive staining for AZGP1 was observed in the large airway epithelial cells in both nonsmokers and smokers. Qualitatively, healthy smokers demonstrated stronger staining and in more cells.

To detect the cell type that was expressing AZGP1, dual immunofluorescence was applied to large airway epithelial cells from a nonsmoker prepared by cytospin using cell type specific marker mucin5AC for secretory cells and chromogranin A for neuroendocrine cells (Fig 6). Secretory cells expressing mucin5AC were readily detected, and a subset of these also expressed AZGP1 (Fig 6, top left, A, to top center right, F). Ciliated cells visible in the same fields never expressed AZGP1. The distinct subcellular distribution of AZGP1 and mucin5AC in conjunction with nonspecific antibody controls (not shown) confirmed the specificity of each antibody. However, not all secretory cells expressed AZGP1 (Fig 6, middle left, G, to middle right, I). Chromogranin A-positive cells were also observed in the cytospin preparation of large airway epithelial cells, and all of these expressed AZGP1 (Fig 6, bottom middle left, J, to bottom right, O). Controls were

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22150/)

![Figure 4](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22150/)

**Figure 3.** Confirmation of the microarray results of AZGP1 expression levels in the large airway epithelium of healthy nonsmokers and healthy smokers. The data included large airway epithelium from 17 healthy nonsmokers and 15 healthy smokers. ordinate: expression level normalized to mean for all nonsmokers ± SEM.

**Figure 4.** Western analysis of AZGP1 protein expression in large airway epithelial cells of healthy nonsmokers and healthy smokers. Top, A (upper panel): AZGP1 protein expression in nonsmokers (lanes 1 to 3) and smokers (lanes 4 to 6). Same gel probed with anti β-actin antibody, a control for protein loading. Bottom, B: quantification by densitometry of AZGP1 to β-actin. The AZGP1/β-actin ratio is represented on the ordinate for the nonsmoker (top, A, upper panel) and smoker bands (top, A). Error bars represent the SE.
performed with matched IgG isotypes to show that the observed signals are attributable to the specific proteins with no cross bleeding between the channels used for detection (not shown).

To quantify the AZGP1-positive cells in non-smokers and smokers, the fraction of AZGP1-positive cells in representative fields of immunofluorescently stained cytospin preparations from 5 nonsmokers and 6 smokers was assessed. Representative low-power views of slides used in the quantitative analysis were shown (Fig 7, top left, A, to bottom middle, D). The mean percentage of positive cells in healthy nonsmokers (6.9 ± 0.7%) was significantly lower than that in healthy smokers (9.9 ± 0.9%; \( p < 0.05 \)) [Fig 7, right, E].

**Discussion**

Cigarette smoking is linked with decreased body weight in smokers, and when smokers stop smoking, they often gain weight. Based on the knowledge that the airway epithelium takes the brunt of the stress of cigarette smoke, we asked this question: does smoking alter the expression of a gene whose product is linked to weight loss? Using microarray analysis to compare gene expression of the airway epithelium in healthy smokers vs nonsmokers, we identified that AZGP1, a gene linked to weight loss, was up-regulated in the airway epithelium of smokers. The microarray data were confirmed at the messenger RNA level by quantitative TaqMan PCR, and at the protein level by immunohistochemistry and Western analysis. We do not have data demonstrating increased levels of AZGP1 in biological fluids, and thus we cannot prove that smoking-induced increases in AZGP1 gene expression in airway epithelium is sufficient to mediate weight loss. However, with the background knowledge that smoking is associated with weight loss, smoking cessation results in significant weight gain from increased body fat, AZGP1 stimulates lipolysis in vitro and in vivo, high systemic levels of AZGP1 are linked to cachexia, and administration of AZGP1 to experimental animals is associated with weight loss, the finding that AZGP1 expression is up-regulated in the airway epithelium in healthy smokers provides another mechanism explaining the weight change that occurs in cigarette smokers.

**Smoking and Body Weight**

Cigarette smokers of the same age and gender weigh less in comparison to nonsmokers, and anorexia is associated with cigarette smoking.
Figure 6. Immunofluorescence assessment of AZGP1 colocalization with mucin 5AC and chromogranin A. Cytospin preparations of large airway epithelium from a healthy nonsmoker were stained with antibodies against AZGP1, mucin 5AC, and chromogranin A, followed by a Cy5-conjugated secondary antibody for AZGP1 (shown in green) and a Cy3-conjugated antibody for mucin 5AC and chromogranin A (shown in red). Nuclei were stained with DAPI (shown in blue); colocalization of AZGP1 with either mucin 5AC or chromogranin A is shown in yellow. Colocalization of AZGP1 and mucin 5AC (panels A to I). Phase contrast image of a cytospin preparation from a healthy nonsmoker showing large airway epithelial cells (top left, A). Top center, B: cytospin preparation from a healthy nonsmoker (same field as in top left, A), but in the Cy5 channel showing AZGP1 staining of large airway epithelial cells. Top left, C: cytospin preparation from a healthy nonsmoker (same field as in top left, A), but in the Cy3 channel showing mucin 5AC staining. Top middle left, D: cytospin preparation from a healthy nonsmoker (same field as in top left, A) showing colocalization of AZGP1 and mucin 5AC (shown in yellow); nuclei are shown in blue. Top middle center, E: cytospin preparation from a healthy nonsmoker (same field as in top left, A) viewed in phase contrast showing colocalization of AZGP1 and mucin 5AC as well as DAPI nuclear staining. Top middle right, F: cytospin preparation from a healthy nonsmoker (same field as in top left, A) demonstrating very minimal to no autofluorescence of large airway epithelial cells. Middle left, G: cytospin preparation from a healthy nonsmoker viewed in phase contrast showing a secretory airway epithelial cell. Middle center, H: cytospin preparation from a healthy nonsmoker (same field as in middle left, G) viewed with the Cy3 and Cy5 channels (stained with both AZGP1 and mucin 5AC antibodies), showing a secretory airway epithelial cell positive for mucin 5AC but negative for AZGP1; nuclei are shown in blue. Middle right, I: cytospin preparation from a healthy
Weight gain is a known deterrent to smoking cessation with an average weight gain of 2.4 to 5 kg.\textsuperscript{12–16} A primary reason smokers give for not trying to quit smoking and for relapsing after cessation is weight gain,\textsuperscript{16,49} and the increase in the prevalence of overeating and obesity in the United States has been attributed in part to smoking cessation.\textsuperscript{50} Weight gain after smoking cessation is largely because of increased body fat.\textsuperscript{12–16} Mechanisms that have been investigated include increased energy intake, decreased resting metabolic rate, and decreased physical activity.\textsuperscript{8,14,19,23,51,52} Studies\textsuperscript{18,53} have not consistently shown increased caloric intake as an explanation for the weight gain following smoking cessation. Nicotine, the major addictive component of tobacco, mediates decreased body weight and food intake in experimental animals and induces lipolysis in smokers.\textsuperscript{22,24,27} This lipolytic effect of smoking is attributed to the effect of nicotine on release of catecholamine, which, in turn, mediates lipolysis in adipocytes.\textsuperscript{1,22,54,55} Some studies have shown that nicotine, as a potent secretagogue in

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**Figure 7.** Quantification of AZGP1-positive cells in healthy nonsmokers and healthy smokers. Cytospin preparations of large airway epithelial cells from five nonsmokers and six smokers were stained with rabbit antihuman AZGP1 or rabbit IgG as control. Goat antirabbit Cy3 was used as a secondary antibody. Nuclei were counterstained with DAPI. The percentage of AZGP1-positive cells was calculated as the number of AZGP1-positive cells per field/total number of nuclei per field in 10 random \( \times 60 \) fields for each subject. Representative low-power (original \( \times 20 \)) staining of specimens from one healthy nonsmoker and one healthy smoker are shown. Top left, A: healthy nonsmoker, rabbit IgG1 isotype control. Top center, B: healthy smoker, rabbit IgG1 isotype control. Bottom left, C: healthy nonsmoker, rabbit antihuman AZGP1 antibody. Bottom center, D: healthy smoker, rabbit antihuman AZGP1 antibody. Bar = 0.01 mm. Right, E: quantification of AZGP1-positive cells; the ordinate shows percentage of AZGP1-positive cells; the abscissa shows the different groups (n = 5 healthy nonsmokers, n = 6 healthy smokers). Error bars represent the SD.
some cell types, mediates the release of peptides that regulate food intake and energy expenditure such as leptin, neuropeptide Y, and orexins.\textsuperscript{25,56,57}

**AZGP1**

AZGP1 was first isolated from human plasma\textsuperscript{33} and later found to be expressed in secretory epithelial cells of the lung, liver, breast, GI tract, and sweat glands.\textsuperscript{38} Consistent with its production by secretory epithelium, AZGP1 is present in most body secretions.\textsuperscript{58,59} Several types of malignant tumors overexpress AZGP1,\textsuperscript{34,60–64} and it has been proposed as a cancer marker.\textsuperscript{34,60–64}

Although the biological functions of AZGP1 have not been fully elucidated, it has been demonstrated to act as a lipid mobilizing factor and is associated with the dramatic weight loss seen in many cancer patients.\textsuperscript{29,34–36} AZGP1 is identified as an adipokine because it is secreted by adipocytes.\textsuperscript{37,65} AZGP1 contains a class I major histocompatibility complex fold and is the sole soluble member of this superfamily of molecules.\textsuperscript{66–68} Uncoupling proteins, members of the mitochondrial carrier family that are postulated to be involved in the control of energy metabolism and body fat, are induced by AZGP1 and cigarette smoke.\textsuperscript{27,28,30–32,66–71} Treatment with AZGP1 stimulates lipolysis in isolated mouse and human adipocytes, and it induces a rapid and selective reduction in body fat both in normal and \textit{db/db} mice.\textsuperscript{35,37} This lipolytic action is mediated via the \(\beta_3\)-adrenoreceptor on adipocytes with up-regulation of cyclic adenosine monophosphate.\textsuperscript{40} Finally, AZGP1-deficient mice have increased body weight when subjected to a standard or high-fat diet when compared to wild-type mice.\textsuperscript{47}

**AZGP1, Cigarette Smoking, and Expression in the Airway Epithelium**

Based on the knowledge that smoking is associated with weight loss, smoking cessation results in weight gain largely from increased body fat, and AZGP1 stimulates lipolysis \textit{in vitro} and \textit{in vivo}, the finding that AZGP1 expression is up-regulated in healthy smokers may reflect one mechanism contributing to the weight changes that occur in cigarette smokers. AZGP1 has been known to be expressed in healthy lung tissue as evidenced by immunohistochemical staining demonstrating AZGP1 expression in airway secretory cells.\textsuperscript{38} A study assessing AZGP1 messenger RNA levels in lung tissue of patients with primary lung cancer and lung metastases revealed no significant difference in AZGP1 levels among smokers and nonsmokers,\textsuperscript{39} but the airway epithelium was not assessed directly, and thus the relationships among lung cancer, smoking, and the expression of AZGP1 in airway epithelium is unclear. AZGP1 was identified in the blood and urine of cancer patients with cachexia,\textsuperscript{34,35} and several studies\textsuperscript{12,75} have supported a role for AZGP1 in cancer. In the present study, the data demonstrate that AZGP1 is significantly up-regulated in the large airway epithelium of healthy cigarette smokers. Interestingly, the immunohistochemistry demonstrated not only expression of AZGP1 in the secretory cells but also in neuroendocrine cells of smokers. The observation that AZGP1 is expressed in neuroendocrine cells may be important in the context that hormonal effects resulting in lipolysis are known to be neurohormonally regulated.\textsuperscript{74}

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