
Tobacco as an allergen in bronchial disease

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Background: Skin testing and sera measurements have verified the existence of tobacco specific IgE. However, the few published studies on this matter report conflicting results concerning their clinical significance.

Objective: To verify if a specific clinical allergenic response against tobacco might be possible in allergenic and nonallergenic bronchial diseases.

Methods: We performed a cross-sectional observational case-control analysis on 180 patients with asthma, chronic obstructive pulmonary disease (COPD), and bronchial carcinoma and controls who were randomly chosen. Skin prick tests and serum specific IgE to tobacco and related allergens, bronchial challenge with cigarettes and tobacco extract, patch tests with tobacco and nicotine, sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting, and Enzyme AllergoSorbent Test (EAST) inhibition were performed.

Results: Twenty-eight patients had positive tobacco skin prick test results. The association among positive skin prick test results, IgE, and bronchial challenge was strong ($P < .001$). Tobacco sensitivity was higher in patients with pollen asthma than in patients with COPD and carcinoma and negative in patients with intrinsic asthma and controls. A positive bronchial challenge result was related to the length of habit ($P < .001$) and the tobacco index in patients who had stopped smoking ($P < .001$). Delayed bronchial and patch response was more common in patients with COPD ($P < .001$). Tobacco IgE response (EAST) was related to sensitivity to *Lolium perenne* (rye grass) pollen ($P < .001$) but not to other vegetables that belong to the Solanaceae family. EAST inhibition showed cross-reactivity between tobacco and *Lolium* pollen.

Conclusions: Tobacco may be responsible for a specific IgE response. Patients with pollen asthma were those with more positive responses to tobacco due to cross-reactivity between *Lolium* and tobacco allergens.

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INTRODUCTION

Asthma morbidity and mortality have increased disproportionately despite the application of new and more active drugs.^{1–5} Chronic obstructive pulmonary disease (COPD) usually affects adult smokers and increases the risk of infection and lung cancer.⁶ In the case of asthma, the airflow limitation is reversible, whereas inflammation is a key characteristic in both diseases.

Enough proof is available to document the strong causal relationship between allergy and the origins of asthma. Susceptibility to both is determined by a combination of genetics and environmental factors that elicit their effects through a complex network of cytokines.⁷ Genetic propensity to asthma and the exposure to tobacco have been shown to be synergic on the risk of childhood asthma.⁸ Nevertheless, results are controversial: although some studies suggest parental smoking may contribute to an airway responsiveness increased as early as the first 2 to 10 weeks of life^{9,10} and maternal

smoking may be associated with an increased incidence of asthma and an earlier onset of disease,¹¹ others have demonstrated a low risk for atopic disorders in smokers themselves and a similar tendency in their children.¹²

Some authors demonstrated that individuals exposed to cigarettes through either active¹³ or passive routes¹⁴ have an increased airway responsiveness. The mechanisms by which tobacco smoke exerts its effect are unknown but may include effects on the IgE immune system that can be activated as early as the intrauterus and postnatal period.¹⁵ Skin testing and serum specific IgE measurements have verified the existence of tobacco specific IgE in some studies¹⁶ and in one case report.¹⁷ Although positive skin response to tobacco is statistically related to atopy and to the presence of environmental tobacco smoke, the low predictive values of skin testing for tobacco hypersensitivity seemed to limit its clinical usefulness.¹⁰ Nevertheless, the possibility of a specific immune response to tobacco warrants a further analysis.

We performed a study on the relationship between environmental tobacco exposure and tobacco hypersensitivity. Patients with asthma and COPD were studied to evaluate if a specific immunologic IgE response to tobacco might be an adjuvant for their bronchial inflammation.

METHODS

Patients and Study Design

The records of 16,381 consecutive patients were studied. These patients had been admitted to our allergy department

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during the last 10 years. The study design was based on a cross-sectional observational case-control analysis. The sample size was calculated by simple random sampling in a ratio of controls to cases of 1, with confidence limits of 95% and a power of 80%.

To homogenize the group of allergic asthmatic patients, only patients with mild asthma caused by grass pollen, the most prevalent allergen in our area, were chosen. Three groups of patients were studied: 60 patients with grass pollen asthma, 60 patients with nonallergy bronchial obstructive diseases (20 patients diagnosed as having COPD, 20 as having intrinsic asthma, 20 as having bronchial carcinoma), and 60 healthy nonsmoking controls. The obstructive pattern was defined by the Tiffeneau index (forced expiratory volume in 1 second [FEV₁]/forced vital capacity [FVC], <70%). None of the control patients were allergic to grass pollen, although 32% had passive exposure to tobacco. To include people never exposed to tobacco, also in a passive manner, 20 of these controls were premature children born in our hospital during the 2 years of the study.

Allergen sensitivity was regarded as the presence of (1) one or more positive skin test results (a wheal diameter of 3 mm or greater than the negative test result) to 37 common allergens, (2) CAP results equal or higher than 0.35 kU/L to these allergens, and (3) a positive specific challenge result.

All these patients were tested to study if tobacco could be a risk factor of bronchial inflammation (tobacco indexes and questionnaires, skin prick tests, specific IgE to tobacco and related allergens, patch tests with tobacco and nicotine, functional respiratory measurements, and immunological tests). They were classified as former smokers, current smokers, passive smokers, and people who had never smoked. Tobacco indexes were defined as follows: number of cigarettes smoked per day $\times$ number of years smoking/20 = pack-years. Those who had stopped smoking at least 12 months before the study were classified as ex-smokers. Written consent was obtained from all patients and controls and in the case of premature children from their parents. Ethical approval was obtained from the Ethics Committee of Rio Hortega Hospital in Valladolid, Spain.

In Vivo Tests

Tobacco extract. Leaves from tobacco were defatted, ground into small pieces, and extracted by magnetic stirring in agitation in 50-mmol/L phosphate-buffered saline at pH 7.5 during 16 hours at 4°C. The sample was centrifuged at 5,600g for 30 minute, and the supernatant was dialyzed against water. The dialyzed extract was filtered through a 0.22- μ m-pore diameter membrane and freeze-dried.

Skin prick test. Skin prick test with extracts from tobacco, potato, tomato, mugwort pollen, *Cynodon dactylon* pollen, and *Lolium perenne* pollen (Bial Aristegui Laboratories, Bilbao, Spain) were performed according to standard procedures² in the volar side of the forearm; one sterile device was used for each test. Tomato leaf and latex extracts were tested because of their possible cross-reactivity with tobacco.^{16,18}

Histamine phosphate (10 mg/mL) and sterile 0.9% saline were used as positive and negative controls, respectively. Skin prick testing with extracts was also performed in a control group of atopic patients.

Bronchial Challenge Tests

Specific bronchial challenges tests (BCTs) were performed after the procedure proposed by Chai et al, with modifications.¹⁹ Aerosolized particles generated by a continuous pressurized nebulizer (Devilbiss 646 nebulizer; Devilbiss Co, Somerset, PA) were inhaled for 2 minutes at normal breathing volume, starting with control phosphate-buffered saline solution, and followed, at 10-minute intervals, by progressive concentrations of the extract. Pulmonary function test results were obtained 30 and 60 seconds after each dose. A positive response was defined as a greater than 20% decrease from basal FEV₁. After specific BCT, hourly peak expiratory flow measurements were recorded for 9 hours.

Three different types of challenge were used. A lit cigarette (10 inhalations) was used before spirometry, but this caused a cough in patients who had never smoked and technical difficulties in pulmonary function tests. Therefore, unlit cigarettes were used to eliminate possibly irritating effects of smoke. The patients were encouraged to face 10 deep breaths before spirometry. Finally, a 1/10 (wt/vol) extract of fresh tobacco leaves was used with a protein concentration of 1 mg/mL to avoid other possible additives of cured tobacco from cigarettes.

Patch Test

Nicotine patch testing was performed using nicotine patch (Nicotinell; Novartis Consumer Health, Uppsala, Switzerland); tobacco patch testing was performed with an extract from a tobacco leaf (1/10 wt/vol) in petroleum jelly. Patch test results were observed at 48, 72, and 96 hours.

Specific IgE Measurements

Determination of specific IgE to all allergens was performed based on the CAP-FEIA technique according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). Specific IgE to tobacco extract was also measured by the Enzyme AllergoSorbent Test (EAST) technique as described by Ceska and Lunqvist.²⁰ Results were expressed in accordance with the manufacturer's instructions (specific IgE EIA kit; Hytec Hycor Biomedical Ltd, Garden Grove, CA). EAST results equal or higher than 0.35 kU/L were considered positive (EAST classes from 1 to 4). A pool of sera from nonallergic individuals was used as the negative control.

EAST Inhibition

EAST inhibition was performed according to the methods reported by Yman et al.²¹ Tobacco and *Lolium* pollen extracts were used as solid phase, and serial 10-fold dilutions (0.0001, 0.001, 0.01, 0.1, 1, and 10 mg/mL) of both extracts were used as inhibitors.

SDS-PAGE Immunoblotting

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli,²² and 12.5% and 4% of acrylamide were used for separating and stacking gel, respectively. Samples were dissolved in 0.125M Tris hydrochloride, pH 6.8, and were dissociated with 0.1% SDS and 5% β -mercaptoethanol at 100°C for 5 minutes. A total of 20 μ g of protein, according to Bradford,²³ was applied per lane.

Separated protein bands were electrophoretically transferred to polyvinylene difluoride as described by Towin et al²⁴ and blocked for 1 hour at room temperature with 5% defatted dried milk in Tris-buffered saline. Membranes were incubated overnight at 4°C with patients' serum samples followed by antihuman IgE–horseradish peroxidase conjugate incubation and detected by the luminescence method as recommended by the manufacturer (ECL-Plus; Amersham Pharmacia Biotech, Buckinghamshire, England).

Statistical Analysis

Data were analyzed using the software package Epi-Info (Stata Corporation, Austin, TX). The normal distribution of variables based on the Kolmogorov-Smirnov test was also studied. χ^2 tests were used to test associations among categorical variables.

RESULTS

The results of all the parameters studied in the different groups of patients can be seen in Tables 1 through 4.

Descriptive Analysis

Anthropometric data. Men (56%) were more numerous than women (44%), and 41% of patients came from urban areas. The average age was 42.18 years (SD, 17.90 years). A total of 12% of our patients worked at home, 10% were farmers, 9% worked in administrative jobs, and 7% were students.

Smoking habits. The proportion of current smokers was 81% and their tobacco index was 28.7 (SD, 24.17). The average length of smoking habit was 20.9 years (SD, 14.06 years). A total of 22% of patients were ex-smokers, of an average of 26 cigarettes per day (SD, 10.79 cigarettes per day), during a mean time of 20.9 years (SD, 14.06 years). The ex-smokers had stopped smoking for an average of 7.4 years (SD, 7.41 years). No differences were found between passive smokers at home and on the job. The average number of cigarettes to which they were exposed was 9.9 cigarettes per day (SD, 4.08 cigarettes per day) during an average period of 14.9 years (SD, 11.67 years).

In vivo tests. Twenty-eight patients (23%) tested had positive skin prick test results to tobacco. Of these patients, 20 were smokers, 7 were ex-smokers, and 1 was a passive smoker. The 60 asthmatic allergic patients were compared with the other 60 patients with nonallergic bronchial disease (20 with intrinsic asthma plus 20 with COPD plus 20 with bronchial carcinoma). Tobacco sensitivity was higher in patients with pollen asthma (18/60; 30%) than in patients with COPD (5/60, 8%) and carcinoma (5/60, 8%) and negative in patients with intrinsic asthma and controls. Eleven patients

Table 1. Characteristics of Patients With Grass Pollen Asthma

Patient No.	Residence	Age, y	Sex	Smoking				Lolium		Tobacco		Mugwort		Latex		Patch		BCT
				Active	Ex	Passive	Non	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Nico-tine	Tobacco	
1	Rural*	21	M	+				10	12	8	7.3	0	0	0	0	0	0	+i
9	Urban	19	M	+				26	0.4	4	0.56	0	0	0	0	0	0	+i
13	Urban	29	M		+			10	29	7	1.69	0	0	0	0	0	0	+i
18	Urban	29	M	+				10	19	8	0.56	0	0	0	0	0	0	+i
22	Rural*	22	M		+			10	23	20	20.8	5	2.8	10	11.2	0	0	+i
28	Urban	23	F	+				24	53	8	7.61	5	2.3	10	52.3	0	0	+i
34	Urban	23	M	+				10	100	8	3.16	6	2.3	0	0	0	+	+d†
38	Urban	20	M	+				30	100	12	1.29	0	0	0	0	0	0	+i
40	Rural*	28	M	+				30	100	10	1.6	0	0	0	0	0	0	+i
41	Urban	37	F		+			20	96	9	4.8	0	0	10	5.8	0	0	+i
43	Urban	32	F	+				30	100	12	8.16	0	0	0	0	0	0	+i
46	Urban	14	M			+		10	0.75	12	5.6	0	0	0	0	0	0	+i
49	Rural*	25	M	+				10	12	12	20.8	0	0	10	18	0	0	+i
50	Rural	63	M	+				10	16.3	5	0.37	0	0	0	0	0	0	+i
51	Urban	43	F	+				12	22	8	0.42	0	0	0	0	0	0	+i
53	Rural*	21	M	+				10	12	8	7.3	0	0	0	0	0	0	+i
55	Rural*	33	M			+		10	21.5	8	5.43	0	0	0	0	0	0	—
58	Urban	33	F				+	10	20	5	5.14	0	0	0	0	0	0	—

Abbreviations: BCT, bronchial challenge test; d, delayed; i, immediate.

Symbols: +, positive; —, negative.

* Farmer.

† Immediate and delayed response.

Table 2. Characteristics of Patients With Intrinsic Asthma

Patient No.	Residence	Age, y	Sex	Smoking				Lolium		Tobacco		Mugwort		Latex		Patch		BCT
				Active	Ex	Passive	Non	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Nico-tine	Tobacco	
101	Rural	20	M			+		0	0	0	0	0	0	0	0	0	0	—
102	Rural*	43	F	+				0	0	0	0	0	0	0	0	0	0	—
103	Urban	48	F			+		0	0	0	0	0	0	0	0	0	0	—
104	Urban	63	M				+	0	0	0	0	0	0	0	0	0	0	—
105	Rural	47	F		+			3	0	0	0	0	0	0	0	0	0	—
106	Rural	28	F	+				0	0	0	0	0	0	0	0	0	0	—
107	Urban	48	F	+				0	0	0	0	0	0	0	0	0	0	—
108	Rural*	36	M		+			0	0	0	0	0	0	0	0	0	0	—
109	Urban	16	F	+				0	0	2	0	0	0	0	0	0	0	—
110	Urban	41	M				+	0	0	0	0.91	0	0	0	0	0	0	—
111	Urban	68	M				+	0	0	0	0	0	0	0	0	0	0	—
112	Rural*	26	M	+				0	0	0	0	0	0	0	0	0	0	—
113	Rural	67	M				+	0	0	0	0	0	0	0	0	0	0	—
114	Urban	49	M	+				3	0	0	0	0	0	0	0	0	0	—
115	Urban	37	F		+			0	0	0	0	0	0	0	0	0	0	—
116	Urban	50	F			+		0	0	0	0	0	0	0	0	0	0	—
117	Rural	59	M		+			2	2	2	0	0	0	0	0	0	0	—
118	Urban	75	F			+		0	0	0	0	0	0	0	0	0	0	—
119	Urban	43	F		+			5	0.7	0	0	0	0	0	0	0	0	—
120	Urban	64	F				+	0	0	0	0	0	0	0	0	0	0	—

Abbreviation: BCT, bronchial challenge test.

Symbols: +, positive; —, negative.

* Farmer.

† Immediate and delayed response.

(9%) were not tested with prick and challenge tests but had positive results on skin patch tests. None of the atopic patients had positive results on skin patch tests to tobacco. Positive nicotine responses were not found. The average values of functional respiratory tests were as follows: FVC, 88.97%; FEV₁, 82.63%; Tiffeneau index, 66.21%; and maximal midexpiratory flow, 39.67%. The results of BCTs with lit cigarettes were positive in 8 (35%) of the 23 patients tested. With unlit cigarettes, we obtained 6 BCT-positive results in 72 patients tested (8%), and with tobacco leaf extract we obtained BCT-positive results in 26 (36%) of 72 patients tested, with 9 delayed responses (12%) (Figure 1).

In vivo tests. The average level of tobacco IgE was 1.05 kU/L (SD, 2.37 kU/L). Because the highest sensitivity to tobacco was obtained in patients with asthma due to *Lolium* pollen, we performed a cross-reactivity study by EAST inhibition assay with these 2 allergens. Three individual serum samples from patients with asthma were used to perform the test. The results were the same with the 3 serum samples: a strong inhibition was obtained when *Lolium* pollen extract was used as the inhibitor and tobacco as the solid phase. On the contrary, tobacco as the inhibitor did not produce any kind of inhibition on *Lolium* pollen (Fig 2). Altogether the results of EAST inhibition indicated that the first sensitization agent in this group of patients was the pollen from *Lolium*:

these patients are allergic to *Lolium* pollen, and some tobacco proteins cross-react with some others of *Lolium*. As a consequence of this, when the immune system of these patients contacts some tobacco proteins, these patients could experience allergic symptoms because of this cross-reaction (tobacco proteins could elicit an allergic response because of the existence of IgE antibodies against *Lolium* proteins, which also react with some tobacco proteins [although with less affinity] because of cross-reactivity).

When tobacco extract is used as solid phase, *Lolium* pollen extract is able to react with all the IgE antibodies that bind with tobacco extract with higher affinity than tobacco extract does, because these antibodies were originally produced against *Lolium* proteins. However, when *Lolium* pollen extract is used as solid phase, only a small fraction of the IgE antibodies (those that cross-react with tobacco proteins) could prevent binding to the solid phase (*L perenne* pollen). This is why tobacco extract seems not to inhibit any IgE binding on *L perenne* pollen; it is able to inhibit some of it, but because it is only a small fraction, it is not reflected in the Figures.

IgE immunoblotting was performed incubating *Lolium* and tobacco extracts with sera from patients sensitized to *Lolium* pollen and tobacco (Fig 3). *Lolium* test results showed an area with a high level of IgE binding between 29 and 100 kDa and a 15-kDa binding band. All sera tested revealed an IgE-binding band of 34 kDa in tobacco extract, and 2 of them

Table 3. Characteristics of Patients With Obstructive Bronchial Disease Due to Bronchial Carcinoma

Patient No.	Residence	Age, y	Sex	Smoking				Lolium		Tobacco		Artemisia		Latex		Patch		BCT
				Active	Ex	Passive	Non	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Nico-tine	Tobacco	
61	Urban	56	M	+				0	0	8	7.10	0	0	0	0	0	+	+i†
62	Urban	74	M		+			0	0	6	6.25	0	0	0	0	0	+	+i†
63	Urban	52	M	+				0	0	0	0	0	0	0	0	0	0	—
64	Rural*	64	M	+				0	0	3	3.82	0	0	0	0	0	+	+d†
65	Urban	70	M		+			0	0	0	0	0	0	0	0	0	0	—
66	Rural*	72	M			+		0	0	0	0	0	0	0	0	0	0	—
67	Rural*	52	M	+				0	0	0	0	0	0	0	0	0	+	—
68	Urban	61	M		+			0	0	2	3.11	0	0	0	0	0	+	+d†
69	Urban	27	M	+				3	0	2	0	0	0	0	0	0	0	—
70	Rural	22	M	+				0	0	0	0	0	0	0	0	0	0	—
71	Rural	52	F	+				0	0	2	0	0	0	0	0	0	0	—
72	Urban	51	M	+				0	0	2	0	0	0	0	0	0	0	—
73	Urban	56	F	+				2	0	8	0	0	0	0	0	0	0	—
74	Urban	77	M	+				0	0	2	3.6	0	0	0	0	0	+	+d†
75	Urban	53	M		+			0	0	0	0	0	0	0	0	0	0	—
76	Urban	26	M	+				0	0	2	0	0	0	0	0	0	0	—
77	Urban	71	F				+	2	0	2	0	0	0	0	0	0	0	—
78	Rural	61	F	+				0	0	2	0	0	0	0	0	0	0	—
79	Rural	43	F		+			2	0	5	0	0	0	0	0	0	0	—
80	Urban	41	M	+				0	0	0	0	0	0	0	0	0	0	—

Abbreviations: BCT, bronchial challenge test; d, delayed; i, immediate.

Symbols: +, positive; —, negative.

* Farmer.

† Immediate and delayed response.

showed a broad and high IgE-binding area ranging from 29 to 100 kDa. In patient 5, the serum level of specific IgE that reacted with the 34-kDa protein was low, and the loss of the native structure of this protein because of the denaturalization process used in this kind of electrophoresis (SDS and high temperature) prevented the binding of any IgE antibody in the blotting assay. In case of the other patient's sera, the level of specific IgE that reacted with the 34-kDa protein should be higher and the denaturalization process should not avoid the binding of some of those IgE antibodies to the protein.

Statistical Analysis

Sensitization to tobacco was found mainly in men ($P < .001$) and farmers ($P < .001$). No relationship was found between the age of patients and sensitization detected by skin prick test, BCT, and specific IgE to tobacco. However, a delayed response in BCT and positive skin patch test results to tobacco was found in older patients ($P < .001$) (14 patients older than 50 years). Negative tests results were significantly associated with nonsmoking ($P < .001$). Delayed responses were more prevalent in urban patients ($P < .03$). The association among positive skin prick test results to tobacco, BCT, and specific IgE to tobacco was strong ($P < .001$).

Positive BCT results were related to the length of habit ($P < .001$) in patients who smoked and the tobacco index in

patients who had stopped smoking ($P < .001$). A delayed bronchial and patch response was more common in patients with COPD ($P < .001$). A positive skin patch test response was associated with the length of habit ($P < .001$) and tobacco index ($P < .001$). In the case of ex-smokers, the negativity of the test results was associated with the number of years since they stopped smoking ($P < .001$).

No significant association was found between the level of specific IgE to tobacco and tobacco index except in the case of ex-smokers in whom an association between the number of cigarettes they smoked and the specific IgE level to tobacco was found ($P < .04$). Tobacco IgE response was related to sensitization to latex ($P < .001$) and pollens from mugwort ($P < .001$) and *Lolium* ($P < .001$) but not to other vegetables that belong to Solanaceae family (potato and tomato). Latex sensitivity and tobacco sensitivity appear to be coincidental, since greater numbers of patients with atopy are more likely to be sensitized to latex.

DISCUSSION

Relatively little information has been published on the influence of current cigarette smoking on asthma morbidity and mechanisms of disease, despite the fact that current asthmatic smokers experience more severe asthma symptoms.²⁵ Recent investigations have suggested that smoking modifies the im-

Table 4. Characteristics of Patients With Chronic Obstructive Pulmonary Disease

Patient No.	Residence	Age, y	Sex	Smoking				Lolium		Tobacco		Mugwort		Latex		Patch		BCT
				Active	Ex	Passive	Non	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Prick test, mm	IgE, kU/L	Nico-tine	Tobacco	
81	Urban	59	M		+			0	0	2	0	0	0	0	0	0	0	—
82	Urban	54	M	+				0	0	5	0	3	0	5	0	0	+	+d
83	Urban	55	M	+				0	0	0	0	0	0	0	0	0	0	—
84	Urban	43	F	+				0	0	0	0	0	0	0	0	0	0	—
85	Urban	70	M			+		0	0	0	0	0	0	0	0	0	0	—
86	Rural*	73	M	+				0	0	8	7.03	0	0	0	0	0	+	+d†
87	Rural*	61	M	+				0	0	10	4.9	0	0	0	0	0	+	+d†
88	Urban	50	F	+				0	0	6	6.05	0	0	0	0	0	+	—
89	Urban	72	M		+			0	0	10	5.39	0	0	0	0	0	+	+d†
90	Rural*	74	M		+			0	0	0	1.57	0	0	0	0	0	+	—
91	Urban	62	M				+	0	0	0	0	0	0	0	0	0	0	—
92	Rural	55	M			+		0	0	0	0	0	0	0	0	0	0	—
93	Rural	65	M				+	0	0	0	0	0	0	10	0	0	0	—
94	Rural*	64	M	+				0	0	8	2.8	0	0	0	0	0	+	—
95	Rural	58	M	+				0	0	0	3.9	0	0	0	0	0	0	—
96	Rural	71	F		+			0	0	0	0	0	0	0	0	0	0	—
97	Rural	77	M		+			0	0	0	0	0	0	0	0	0	0	—
98	Rural	64	M	+				0	0	0	0	0	0	0	0	0	0	—
99	Rural	68	M		+			2	0	5	0.79	0	0	0	0	0	+	+d†
100	Rural	43	M		+			0	0	0	0	0	0	0	0	0	0	—

Abbreviations: BCT, bronchial challenge test; d, delayed; i, immediate.

Symbols: +, positive; —, negative.

* Farmer.

† Immediate and delayed response.

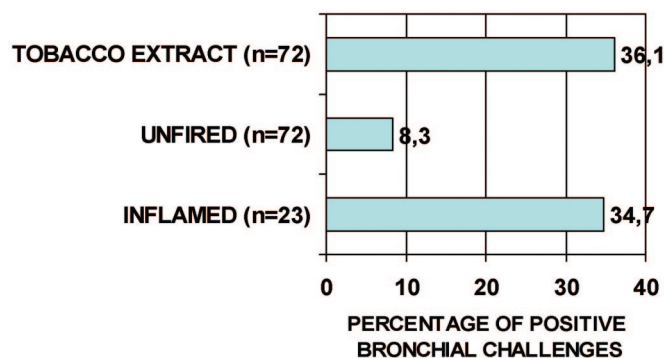


Figure 1. Bronchial challenge test results.

munological response to asthma.^{19,26} Nevertheless, the studies published so far report conflicting results concerning the clinical significance of tobacco as an allergen.

We demonstrate the possibility of a specific clinical IgE and delayed immune response to tobacco as a specific allergen that may have influence on different bronchial obstructive conditions. We also demonstrate cross-reactivity between tobacco and *Lolium* (rye grass pollen), which may be important for asthmatic patients with hypersensitivity to this pollen.

Cigarette smoking may modify airway inflammation associated with asthma, reducing eosinophil counts¹⁸ and inter-

leukin 18 levels in sputum,²⁷ altering the balance of T_H1/T_H2 cytokine secretion, and increasing neurogenic inflammation,²⁸ oxidative stress,²⁹ and airway remodeling.³⁰ Cross-reacting allergens have been described among tobacco, cocoa, coffee, and ragweed pollen,³¹ capable of activating factor XII-dependent pathways. A glycoprotein antigen has been isolated from tobacco leaves (*Nicotiana tabacum*) and tobacco glycoprotein from cigarette smoke condensate to which approximately one third of human volunteers, smokers and nonsmokers, exhibit immediate cutaneous hypersensitivity. Tobacco glycoprotein is also capable of complement activation through the classic pathway³²; therefore, complement activation may be another type of tobacco-induced inflammation.

The possibility of tobacco as a specific allergen on airway inflammation was perhaps underestimated. Eggleston et al¹⁹ have suggested that atopy might influence the interaction between smoking and asthma. In the general population, current cigarette smoking is associated with the risk of developing occupational asthma due to IgE-inducing agents.³⁰ In this way, tobacco can be a possible source of vegetal allergens that may also cause extrinsic asthma, as the results of our in vivo tests support (positive skin prick test and BCT results with tobacco leaf extract). Nevertheless, only their role as occupational allergens has been described.^{16,17} In our study, those patients with asthma due to pollen showed high

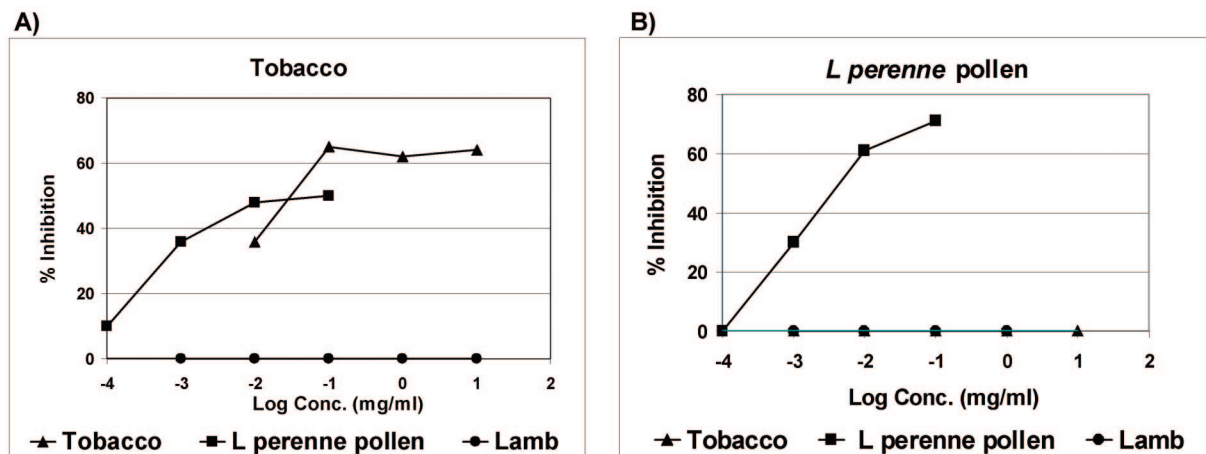


Figure 2. Enzyme AllergoSorbent Test inhibition. Results obtained with a representative serum. A, Tobacco extract as solid phase. B, *Lolium perenne* pollen extract as solid phase. Conc. indicates concentration.

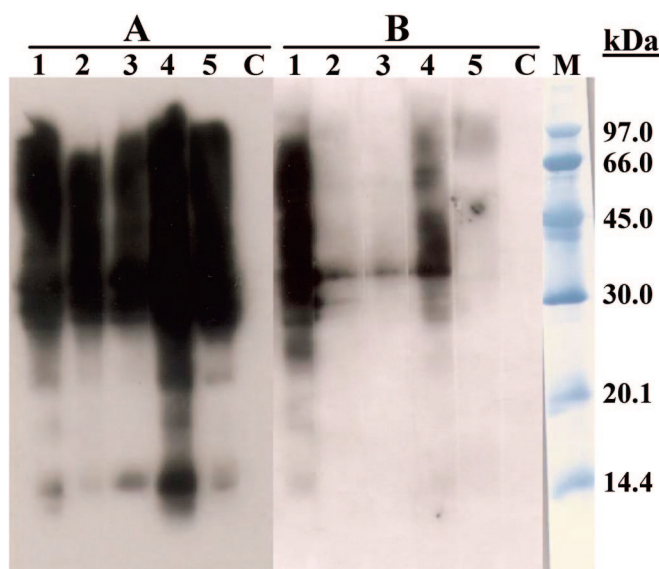


Figure 3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis IgE immunoblotting results. A, *Lolium perenne* pollen extract. B, Tobacco extract. Representative sera from sensitized patients to both *L perenne* pollen and tobacco extracts.

levels of sensitivity to tobacco. It is possible that the atopic status might influence the immunological effects of active smoking when these patients handled or smoked cigarettes. In this respect, IgE sensitization was possible by passive exposure in one patient. No evaluation of tobacco smoke captured by filtration was performed, but positive results of BCT with unlit cigarettes were obtained.

Our results raise an important issue of whether airway inflammation in cigarette smokers who have allergic asthma differs from that of patients with nonatopic asthma and other bronchial obstructive conditions. Together with the fact that

IgE sensitivity is more common in atopic individuals, our results suggest that in patients with COPD a specific sensitivity to tobacco is also possible with more delayed response. A positive skin patch test result and delayed response in BCT was the more common patron response found in patients with COPD, those with carcinoma, and older patients, which suggests the implication of chronic inflammation mechanisms. The small number of patients with carcinoma and COPD due to difficulties in patient participation limits the results of our study, but it is possible that, in the first stages of their illness, some patients had extrinsic asthma that over time presented the characteristics of COPD. The possibility of sensitivity is not dependent on the tobacco index in extrinsic asthmatic people, but in patients with COPD positive responses were associated with the length of habit and the number of cigarettes that they smoked. In this way, the negativity of the test results was associated with the number of years since they had stopped smoking, which supports the advisability of stopping smoking as soon as possible.

Another observation that deserves a comment is the higher sensitivity in atopic farmers, which may be because they used tobacco as insecticide against crow pests. On the other hand, it is also possible that sensitivity to tobacco in atopic patients could be due to cross-reactivity with other vegetal allergens. The different results obtained in EAST inhibition tests when tobacco or *Lolium* extract were used as inhibitors lead us to suppose that patients were primarily sensitized by *Lolium* allergens and then, because of cross-reactivity, to tobacco proteins that react with specific *Lolium* IgE.

In summary, we have demonstrated that tobacco may be responsible for a specific IgE response. In our study, patients with asthma due to pollen had the most positive responses to tobacco. We have therefore demonstrated cross-reactivity between pollen and tobacco allergens. Our results reinforce the importance of stopping smoking in patients with asthma to better deal with their condition.

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