

Short communication

Sensitization to the fungus *Hemileia vastatrix* (coffee leaf rust)

Background: The aim of the present study was to determine the existence of sensitization to the fungus *Hemileia vastatrix* (coffee leaf rust) in a sample of the Brazilian population. This fungus attacks coffee plantations, and in some regions of Brazil the concentration of its spores in the air can be very high.

Methods: A total of 378 individuals underwent skin tests (prick and intradermal tests) with *H. vastatrix* extract. The subjects were divided into four groups according to the occurrence of atopy and the region where they lived (coffee-growing or non-coffee-growing regions), and another group (V) consisted of 50 rural workers employed on coffee plantations. The presence of specific IgE against the fungus *H. vastatrix* in sensitized individuals was demonstrated *in vitro* by immunoblotting.

Results: There was no statistical difference in comparing the results with intradermal or prick tests. The incidence of positive tests was significantly higher among atopic individuals residing in coffee-growing regions (14.7%). Among rural workers, 10% showed positive tests. Immunoblotting revealed specific IgE against 20 protein bands of *H. vastatrix*.

Conclusions: The fungus *H. vastatrix* may be an important allergen in coffee-producing countries. The sensitization is more frequent among atopic individuals residing in coffee-growing regions.

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Among allergens, the fungi present the widest variation from one environmental region to another. Until the 1970s, only fungi of the subdivision Deuteromycotina were recognized as allergens. The first reports identifying basidiomycetes as agents causing asthma were published by Salvaggio et al. (1, 2), and the existence of allergy to several basidiomycete species has been demonstrated since then (3–9).

Some rusts, such as cocoa rust, sugar rust, and coffee rust, specifically parasitize one vegetal species. The basidiomycete *Hemileia vastatrix*, which belongs to the subdivision Basidiomycotina, family Pucciniaceae, order Uredinales (rusts), causes orange coffee leaf rust. The fungus attacks the leaves of the coffee plant, producing pale yellow spots on their dorsal surface. In Brazil, currently the major coffee-producing country, coffee leaf rust was first detected in 1970 in plantations in the southeast region (10).

The average diameter of *H. vastatrix* spores is 5 µm; its sporulation occurs from May to October, the cold months in Brazil. Large numbers of spores are released

into the atmosphere, reaching heights of more than 1000 m (11). It was observed that the incidence of asthma attacks and rhinitis increases during the winter in the southeast region (12).

The present study aimed to determine the incidence of sensitization to this fungus in a sample of the Brazilian population. Appropriate extracts of *H. vastatrix* spores for skin testing were obtained, and the existence of specific IgE against the fungus *H. vastatrix* in sensitized individuals was demonstrated *in vitro* by immunoblotting.

Material and methods

Preparation and standardization of extract

Uredospores of the fungus *H. vastatrix* were directly collected from coffee (*Coffea arabica*) leaves contaminated with the fungus by scraping the mycotic lesions with a metal blade. Extract was prepared in a 10% proportion (uredospores weight times total volume) with phenol-free Coca solution at pH 8.0 as extracting substance. Briefly, the collected material was washed and dried. The

spore mass was grained and mixed with the extracting solution under constant shaking at 4°C for 7 days. After centrifugation, the supernatant was submitted to dialysis with distilled water and 0.1 M ammonium carbonate. The extract thus obtained was submitted to bacteriologic tests to ensure sterility.

The extract was analyzed for carbohydrate, protein, and lipid content by the appropriate methods (13–15). The proteins in the crude extract were fractionated and characterized by polyacrylamide gel electrophoresis (SDS–PAGE) by the method of Towbin et al. (16) with low-molecular-weight standards (Calibration kits, LMW 14 000–94 000, Pharmacia Laboratories, Uppsala, Sweden). *H. vastatrix* extract was lyophilized and later reconstituted with deionized water to obtain protein contents of 2 mg/ml for the skin prick test and 0.02 mg/ml for the intradermal test.

To determine the sensitivity to other allergens and atopic statement, prick tests were performed with extracts of *Dermatophagoides pteronyssinus* (D pt), dog epithelium, cat epithelium, and a mixture of anemophilus fungi (*Alternaria* sp., *Chaetomium* sp., *Cladosporium favum*, and *Cladosporium herbarum*) (ALK-Abelló Laboratory, Spain).

The skin prick tests were performed by the technique of Pepys (17). Measurement of a wheal 3 mm larger than the negative control or a wheal measuring more than 5 mm was considered positive. In the intradermal test, 0.01 ml of the extract was injected by the intradermal route. The test was considered positive if the widest diameter of the erythema plus the diameter perpendicular to the midpoint of that was greater than 50 mm (D50) (18).

Study population

A total of 378 individuals underwent skin prick test, and 321 individuals underwent the intradermal test with *H. vastatrix* extract. The subjects were divided into four groups according to the occurrence of atopy and the region where they lived (coffee-growing or non-coffee-growing regions). Another group (V) consisted of 50 rural workers employed on coffee plantations (Table 1).

The ethics committee of the Hospital of the University of São Paulo (Brazil) approved the study, and the subjects signed a consent form after being informed of the objectives and risks of the study. A clinical history of asthma and/or rhinitis triggered by allergens and a positive skin test to one or more common environmental allergens were considered to be criteria indicating the presence of atopy.

Detection of specific IgE

The immunoblotting technique was used to test serum samples from 17 individuals with positive skin tests and three asymptomatic individuals with negative skin tests.

Statistical analysis

Data were analyzed statistically by the chi-square test, with the level of significance set at $P < 0.05$.

Results

Biochemical evaluation of crude extract

The 10% *H. vastatrix* crude extract obtained with Coca solution contained 0.087 mg of proteins/ml, 0.931 mg of carbohydrates/ml, and 0.22 mg of lipids/ml.

Fractionation and protein characterization

Electrophoresis of the crude extract revealed countless protein bands of molecular masses ranging from 12 to 97 kDa.

Table 1. Characteristic of groups submitted to skin test with *H. vastatrix*

Group	No. of subjects	Sex M/F	Origin in coffee-growing region	Atopic
I	102	39/63	Yes	Yes
II	57	31/26	Yes	No
III	118	55/63	No	Yes
IV	51	12/39	No	No
V	50	49/1	Yes	No
Total	378	186/192	209	220

Skin tests

The number of individuals presenting positive skin prick tests and intradermal tests with the extract is shown in Table 2. When the results of the skin prick tests and intradermal tests were compared, no significant differences were observed.

Coincidence in sensitization among the groups studied

When the results of the skin tests were analyzed with prick tests as reference, comparison with the results obtained with the intradermal test showed 82–96% coincidence of positive results in the same individuals in the five groups studied.

Difference in sensitization among the groups studied

The results with the prick tests showed a significant difference between group I and group IV ($P = 0.034$) and between group I and group III ($P = 0.040$) (chi-square test) (Table 2). Thus, significant differences in the incidence of sensitization to the fungus *H. vastatrix* were observed between atopic individuals from coffee-growing regions (group I) and both atopic and nonatopic individuals from other regions (groups III and IV). Rural workers employed on coffee plantations (group V) did not present a significant sensitization compared to individuals residing in the same region or in others. The same analysis done with the intradermal tests showed similar results.

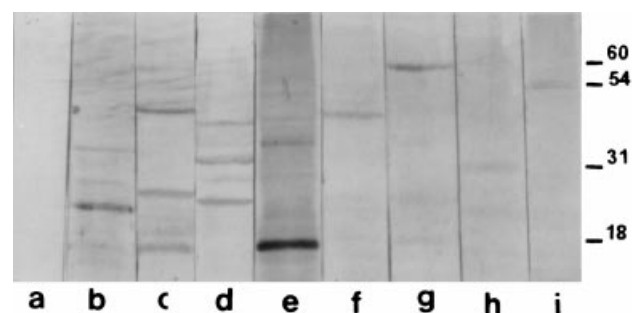


Figure 1. Specific IgE for *H. vastatrix* detected by immunoblotting. Lane a) control individuals; lanes b–i) individuals with positive test.

Table 2. Number of positive skin tests to *H. vastatrix* extracts in each group

	Prick*	Intradermal**
Group I	14 (14.72%)	16 (14.72%)
Group II	5 (8.77%)	ND
Group III	7 (5.93%)	6 (5.08%)
Group IV	2 (3.92%)	2 (3.92%)
Group V	5 (10%)	6 (12%)
Total	33 (8.73%)	30 (9.34%)

* Protein concentration: 2 mg/ml.

** Protein concentration: 0.02 mg/ml.

Total number: 321.

ND: not done.

Detection of the allergenic fractions of *H. vastatrix* extracts

Specific IgE against 20 protein fractions in the *H. vastatrix* extract were detected in the sera of 17 individuals with positive skin tests. In contrast, the sera of the three individuals with negative skin tests did not present IgE reacting with fungal proteins. Fig. 1 shows the reactions of eight sera from individuals with positive skin test (b–i) and one serum from an individual with negative skin prick test (a).

Discussion

The crude extracts obtained of *H. vastatrix* uredospores showed a low protein concentration, as seems to be characteristic of most basidiomycete extracts (5). To obtain basidiomycete extracts with higher protein concentrations, it has been recommended that extraction be performed with a bicarbonate substance (7, 9). Therefore, to perform the skin tests, it was necessary to concentrate extracts. After several assays, we chose the protein concentrations of 2 mg/ml for the prick test and 0.02 mg/ml for the intradermal tests (data not shown). At these concentrations, we obtained similar results both in the prick and in the intradermal tests and a high coincidence of positive results in the same individual.

Another characteristic observed was the large amount of carbohydrates in the *H. vastatrix* extracts. The analysis of these carbohydrates identified many lectins, which are necessary for adhesion of the spores

to the coffee plant (20). Because of the presence of lectins, it is possible that nonallergic skin reactions may occur. We think this was unlikely to have occurred because these nonspecific reactions would involve a large number of individuals regardless of their atopic characteristic or the region in which they live. Furthermore, we demonstrated *in vitro* the presence of a specific IgE for *H. vastatrix*, a fact supporting the hypothesis of an allergic mechanism.

Immunoblotting revealed IgE that reacted with several protein bands of the *H. vastatrix* extract. It was not possible to identify a major allergenic fraction, a process that would require the study of a large number of sensitized individuals. It must be considered that fungal extracts usually present a large number of protein bands (21).

Another consideration is the existence of cross-reactions among basidiomycetes (6, 8, 22–25). There are few studies of other species of basidiomycetes in southeast Brazil. The country has large areas devoted to cocoa and sugar-cane plantations, which may also be attacked by rusts. Cross-reactions between these basidiomycete species may occur, increasing the number of positive tests in the population.

Our results show that the incidence of sensitization to coffee leaf rust varied from 3.92% to 14.72% according to the group studied, reaching the highest indices among atopic individuals from coffee-growing regions. Analysis of the results showed a statistically significant difference between atopic individuals residing in coffee-growing regions and individuals from other regions. Individuals may have been sensitized by inhalation of spores directly or eventually when traveling through the country. *H. vastatrix* may be an important allergen in Brazil and other coffee-producing countries.

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