

Occupational sensitization to *Plasmopara viticola*

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Abstract

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Molds of the class of *Oomycetes* are of allergologic importance in special cases. However, probes are not commercially available for diagnostic purposes. Our case report is based on the medical history of a greenhouse worker who had atopic syndrome. He handled pure cultures of defined fungi and plants. A sensitization to pseudo mildew growing on grapevine (*Plasmopara viticola*) was found. Skin prick test and histamine release test results were positive when extract of *P. viticola* was used. Detection of IgE reactivities against pseudo mildew via binding tests, Western blot analysis, and isoelectric focusing immunoblot confirmed the diagnosis. To our knowledge these results demonstrate for the second time a sensitization to *Oomycetes*. (J ALLERGY CLIN IMMUNOL 1994;93:457-63.)

IEF	Isoelectric focusing
RLU	Relative light units
SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SU	Standard units

Key words: [Plasmopara viticola](#), [pseudo mildew](#), [Oomycetes](#), [occupational allergy](#), [IgE](#), [histamine](#)

Molds are known to cause type I and type III hypersensitivity. In this context the *Deuteromycetes* are the most important subdivision because they include the allergologically relevant and well known genera such as *Alternaria*, *Aspergillus*, *Auerobasidium*, *Cladosporium*, and *Penicillium*.¹⁻⁴ *Mucor* and *Rhizopus*, derived from the class of *Zygomycetes*, are relevant allergens, too.^{5, 6} In general the class of *Oomycetes* is of little importance from the allergologic point of view.^{5, 7} *Pythium* species of this class causes pythiosis in dogs, horses, and human beings.^{8, 9} In this article we describe a patient with a sensitization against the pseudo mildew of grapevine, *Plasmopara viticola*, which belongs to the class of *Oomycetes*. In wet weather conditions this parasite grows epidemically on the leaves of grapevines and tends to destroy every year a great amount of the vintage.

METHODS

Case report

The patient is a 31-year-old agricultural technician, specializing in the development of fungicides for agriculture. His work requires infecting cultivated plants with parasitic fungi and testing the effects of fungicides. He performs his experiments in a greenhouse at air temperatures in the range of 20° to 25° C and relative humidity up to 70%.

Thus he was exposed to fungi by inhalation and skin contact for 7 years. He studied *Botrytis*, *Helminthosporium*, and *Phoma* in detail and less frequently *Alternaria*. He experimented with mildew of grapevine (*Uncinula necator*), mildew of barley and wheat (*Erysiphe*), and mildew of apple (*Podosphaera leucotricha*), and *Puccinia recondita*. Among the class of *Oomycetes* that the patient tested was the pseudo mildew on grapevine, *Plasmopara viticola*.

Our patient was known to have sinusitis, headaches, faintness, difficulties in breathing, and eczema of the hands. The first work-related symptoms, rhinitis and dyspnea, appeared about 4 years ago. The patient reported that rhinorrhoea, sneezing, and dyspnea decreased obviously on holidays and noticeably on weekends. He believed that his respiratory problems increased when he was working intensively with *Plasmopara viticola* or *Alternaria* species. However, he studies *Alternaria* species rarely.

The patient had undergone hyposensitization therapy with extracts of grass pollen (*Phleum pratense*) and rye pollen. He experienced no improvement in his respiratory symptoms after this therapy.

The physical examination and medical history produced unobstrusive results, apart from eczematous altered hands. Lung function analysis revealed static and dynamic volumes and bronchial resistance within the normal range. The results of the standard prick test

indicate a sensitization to tree and grass pollen, rye pollen, cat, and *Alternaria*, *Aspergillus*, *Cladosporium*, *Neurospora*, *Mucor*, and *Penicillium*. The RAST showed the following results: *Alternaria tenuis* (RAST class 2), *Helminthosporium halodes* (RAST class 1), *Fusarium moniliforme* (RAST class 1), *Phoma betae* (RAST class 1), *Aspergillus* (RAST class 0), *Mucor racemosus* (RAST class 0), and *Botrytis cinerea* (RAST class 0).

These tests were performed when the patient was 31 years old. The patient did not want to change his work place, but he used a particle filter at work thereafter. He reported a noticeable but not complete decrease in his respiratory symptoms since he began using the mask.

Control groups

There were four different control groups. Group 1 was a 51-year-old coworker at the same greenhouse. He had no history of allergies (total IgE < 5 IU/ml). For group 2, 10 of 112 workers from an institute for wine growth (mean age: 41 ± 13.8 years; 8 men and 2 women; total IgE = 144 ± 189 IU/ml) were selected. All were exposed to molds of grapevine in their professions. Subjects had eczema (four), chronic urticaria (three), or chronic bronchitis (one). Group 3 comprised three men, aged 31, 33, and 45 years, who were not exposed to molds in greenhouses or agriculture and who had no bronchial hyperreactivity (total IgE = 178 ± 120 IU/ml). Group 4 comprised two men, a 28-year-old with atopic asthma and a 33-year-old control subject with bronchial hyperreactivity. Both were not exposed and not sensitive to pseudo-mildew (total IgE = 220 ± 212 IU/ml).

Allergen extracts

Selected greenhouse-cultured plants were infected with *Plasmopara viticola*, *Podosphaera leucotricha*, *Erysiphe graminis hordei*, *Erysiphe graminis tritici*, *Uncinula necator*, or *Puccinia recondita* (rust). From these plants the mycelia and spores of the fungi were stripped mechanically from the leaves with 0.1 mol/L ammonium carbonate at pH 7.85 as rinsing solution. The fungi were homogenized (Stirrer RW 25, Ika Werk, Staufen, Germany; tissue grinders, type Potter-Elvehjem, Wicker & Löscher GmbH, Kirchheim u. Teck, Germany) and centrifuged at 4° C for 30 minutes at 30,000 g. The supernatant was freeze-dried and stored at -20° C.

Skin prick test

The freeze-dried fungi extracts were solubilized in 0.9% NaCl, sterilized by filtration through a 0.45 µm filter (Milles-HA 0.45 µm, Fa. Millipore, Molsheim, France). The skin prick tests were administered in five concentrations: 0.5 µg/ml, 5 µg/ml, 50 µg/ml, 0.5 mg/ml, and 5 mg/ml. We used 0.9% NaCl and histamine as control. The results were considered positive if the average of wheal diameters was 5 mm or greater. The five volunteers in control groups 3 and 4 and the patient were tested. In control subjects only minimal irritative reactions (wheal < 2 mm) could be observed.

Chemiluminescence immunoassay technique

Specific human IgE antibodies were estimated by the chemiluminescence immunoassay technique with the Magic Lite Analyzer II (Ciba Corning, Fernwald, Germany).¹⁰ We used a self-made solid phase. Polystyrol tubes (Maxisorp Star Tubes, Nunc, Wiesbaden, Germany) were coated overnight with 200 µl of crude extract of *Plasmopara viticola*,

Uncinula necator, or *Podosphaera leucotricha* in 0.025 mol/L sodium carbonate-bicarbonate buffer (pH 9.6) at various dilutions (1 mg/ml, 0.1 mg/ml, 0.01 mg/ml). Tubes were saturated with 500 µl incubation buffer. After two washes (500 µl washing solution), the tubes were incubated with 200 µl of diluted serum (1:4 in incubation buffer) overnight at 4° C.

The tubes were washed three times, and allergen-specific IgE antibodies were detected with 100 µl of mouse anti-human antibody labelled with acridinium ester as the chemiluminescent compound (Ciba Corning) together with 100 µl of 0.9% saline solution.

After 1-hour incubation at room temperature followed by three washes, 100 µl of water was added, and bound acridinium ester-labelled antibody was measured with the Magic Lite Analyzer II (Ciba Corning), which automatically injects the hydrogen peroxide and sodium hydroxide (reagents 1 and 2, Ciba Corning) necessary to start the chemiluminescent reaction.¹⁰ The released photons were expressed as relative light units (RLU).

All assays were performed in duplicate. We used individual serum samples from the patient and all control subjects of groups 1 to 4 and buffer solution as negative controls. Washing buffer consisted of 50 mmol/L Na₂HPO₄/NaH₂PO₄ (Ph 7.4), 150 mmol/L NaCl, 0.1% Tween 20, 0.1% bovine serum albumin. Incubation buffer was made with the same ingredients as the washing solution, but 0.5% bovine serum albumin was used.

Isoelectric focusing immunoprint

Proteins (12.5 µg/cm) were separated by isoelectric focusing (IEF) and transferred by capillary action to CNBr-activated nitrocellulose membrane (BA 85, pore size 0.45 µm; Schleicher & Schüll GmbH, Feldbach, Switzerland) by means of the IEF print technique.¹¹ The CNBr-activated nitrocellulose was prepared according to the method of Demeulemester et al.¹² IEF was performed in 0.5 × 220 × 100 mm thin-layer agarose gels (0.9% wt/vol agarose IEF; Pharmacia, Uppsala, Sweden) containing 3% wt/vol Servalyt 3-10 and 1% wt/vol Servalyt 4-6 (Serva Feinbiochemica GmbH & Co., Heidelberg, Germany). As gel support, GelBond film (Pharmacia) was used. The running conditions of IEF were as follows: time: 1200 Vh, U_{max}: 2000 V, I_{max}: 20 mA, P_{max}: 35 W, setting of the coolant: 0° C where U is voltage, I is current, and P is power.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis blot technique

Proteins (12.5 µg/cm) were separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in 0.5 mm wide gels and transferred electrophoretically by semi-dry blotting.¹³ For discontinuous SDS-PAGE the buffer system of Neville and Glossmann was used.¹⁴

Detection of proteins and antigens

After blotting, the nitrocellulose membrane was incubated in 0.1 mol/L Tris-buffered saline (pH 7.4) containing 0.05% (vol/vol) Tween 20. (This buffer is also used for washing steps and for the dilutions of antibodies.) For investigations the membrane was cut into strips. For protein staining the strips were incubated in 1% (vol/vol) India ink (Pelikan AG, Hannover, Germany).¹⁵ For the detection of allergens, the strips were incubated with patients' or control subjects' sera (1:20) overnight. All subjects in control groups 1, 2, and 3 were tested. Afterwards, the nitrocellulose strips were put into alkaline phosphatase–

conjugated goat-anti-human IgE (Medac, Hamburg, Germany), diluted 1:1000, for 2 hours. The binding patterns were made visible by the substrate solution of 0.1 Tris-buffered saline (pH 9.5) containing 0.033% (wt/vol) nitro blue monotetrazolium chloride and 0.017% (wt/vol) 5-bromo-4-chloro-3-indolyl phosphate potassium salt (Serva).¹⁶

Histamine release test

The histamine release test was performed in 1 ml diluted (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer pH 7.4, 1:1) heparinized whole blood of the patient and the exposed coworker (control subject 1). One milliliter of diluted blood without allergen served as negative control.¹⁷ Tests with anti-IgE, protein content 15 µg/L (Dakopatts, Hamburg, Germany) served as positive controls. Twenty microliters of the fungi extracts (concentrations from 0.5 µg/ml to 5 mg/ml in ten-fold increasing steps) were applied to the reaction tubes to initiate histamine release. They were incubated for 20 minutes at 37° C. After centrifugation, the supernatant was immediately stored at –20° C and measured by means of a radioimmunoassay (Immunotech supplied by Biermann, Bad Nauheim, Germany), which includes an acetylation step with histamine.¹⁸

Bronchial challenge test

The patient and the control subjects in group 4, one with asthma and the other with bronchial hyperreactivity, underwent nonspecific and specific bronchial provocation tests. The nonspecific bronchial provocation test with acetylcholine was used to determine the presence of bronchial hyperreactivity. We performed the four-step acetylcholine test as described by Schwaiblmair et al.¹⁹ After saline control challenge, values of bronchial resistance increased not more than 15% above baseline levels. Acetylcholine was nebulized with a Pari provocation test I instrument (Pari-Medanz, Starnberg, Germany). Particles with an aerodynamic mass median diameter of 2 to 3 µm were inhaled via the reservoir method described by Klein et al.²⁰ The applied acetylcholine doses of the four steps were 0.5 mg, 1.5 mg, 5 mg, and 15 mg; the interval between steps was 5 minutes.

The specific bronchial provocation test with extract of *Plasmopara viticola* was performed to confirm or exclude the bronchial manifestation of the sensitization to this allergen. This test was performed 12 weeks after acetylcholine testing. The patient was removed from the workplace 2 days before the bronchial challenge test.

Freshly prepared solution of freeze-dried extract of *Plasmopara viticola* was passed through a Millipore filter (Milles-HA 0.45 µm; Millipore) for sterilization and was nebulized by the Pari provocation test I instrument as described above. The cumulative applied dose was 555 mg of the extract.

The bronchial reaction is considered positive if the bronchial resistance increases by 100% or more. The patient and the control subjects had not been receiving any drug therapy for more than 3 weeks before testing.

RESULTS

Skin test

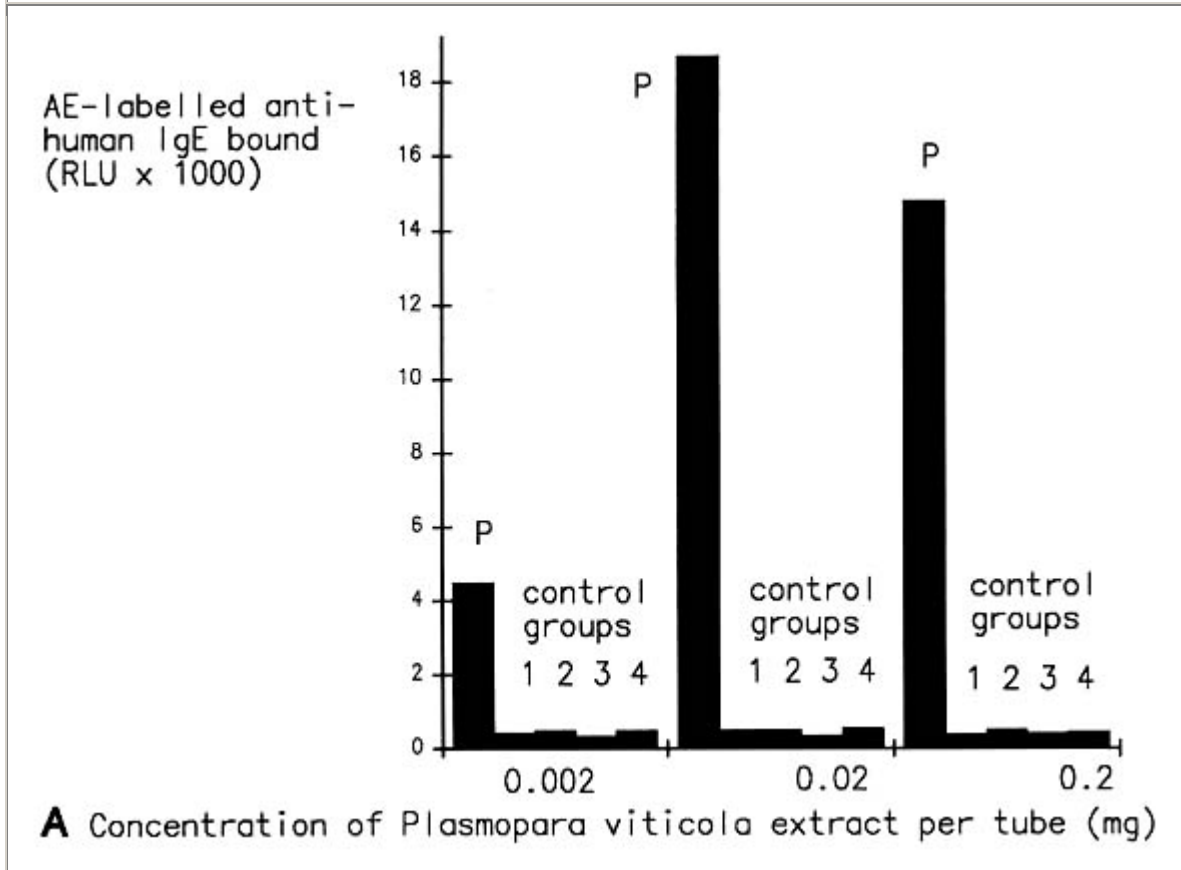
In the patient, *Plasmopara viticola* caused an immediate positive response in a dose-

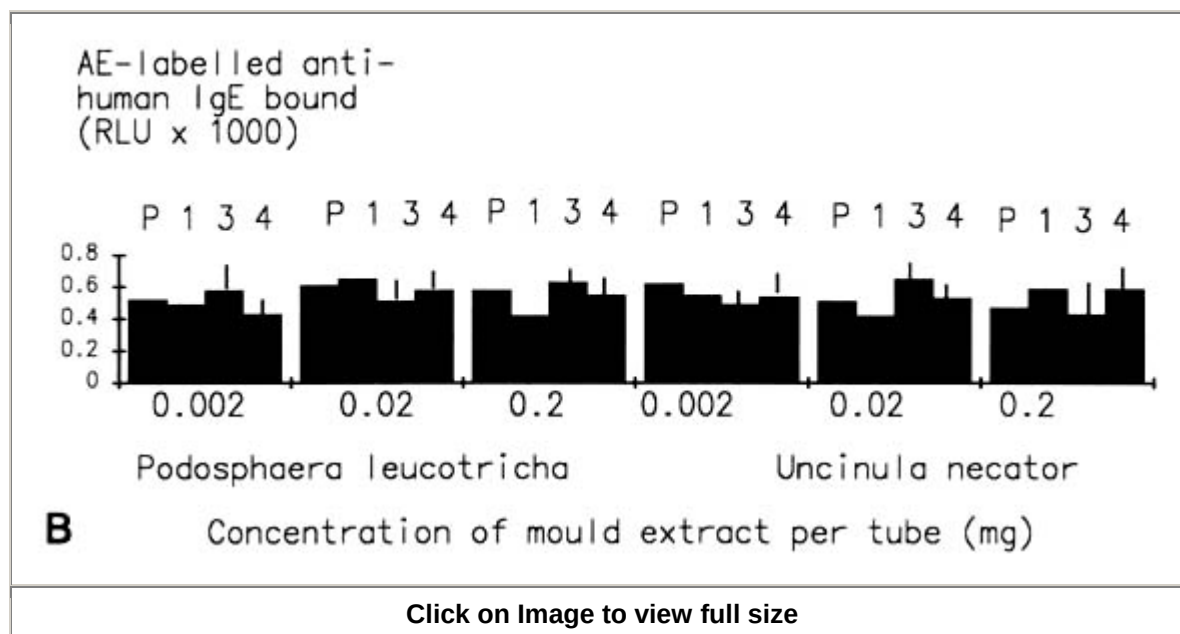
dependent manner at a concentration of 0.5 µg/ml (7 mm wheal) and 5 µg/ml (12 mm wheal). The extracts of the mildew and *Puccinia recondita* caused an irritative reaction, if at all, (average wheal diameter < 2 mm). All control subjects had negative skin reactions to the extracts of these molds (average wheal diameter < 2 mm). Histamine results were positive in all subjects.

Chemiluminescence immunoassay technique

Serum from the patient and from all subjects in control groups 1 to 4 were tested. Coating with 20 µg crude extract per tube produced the best results (■Fig. 1). We obtained a value of 18,690 RLU in patient's serum from bound acridinium ester-labelled anti-human IgE (■ Fig. 1, P). In control sera the values were in the range of 300 to 700 RLU (■Fig. 1, A and B).

FIG. 1. Results of the chemiluminescence immunoassay. **A**, Tubes were coated with 0.002, 0.02, and 0.2 mg crude extract of *Plasmopara viticola*. **B**, Tubes were coated with 0.002, 0.02, and 0.2 mg crude extract of *Podosphaera leucotricha* or *Uncinula necator*. P represents the greenhouse worker; and 1, 2, 3, and 4 represent the control groups.





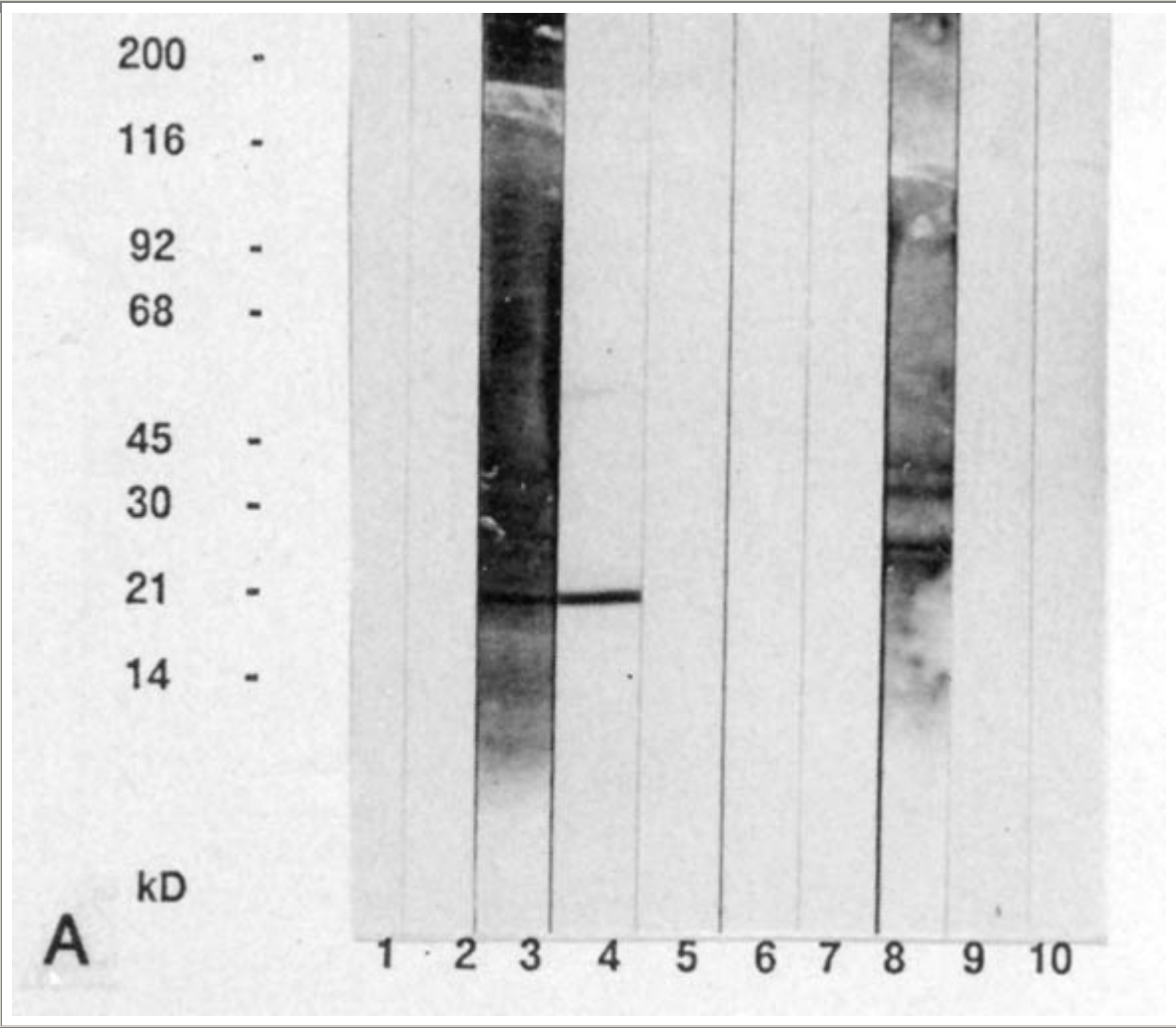
Quantitative interpretation

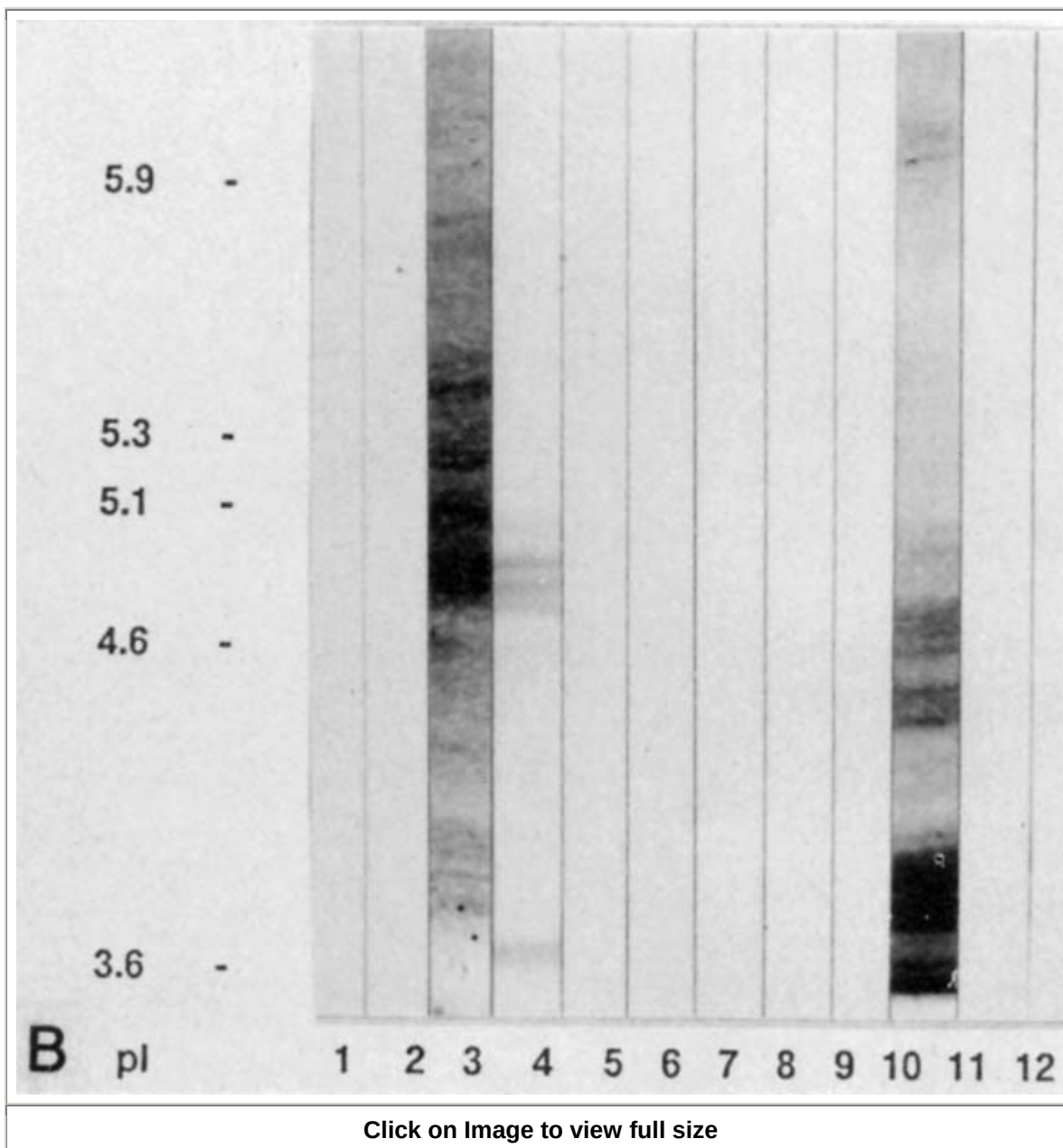
With the use of commercially available reference standards for evaluation of allergenic reactivity (standard units [SU] per milliliter) in the Magic Lite Standardized Quality Specific IgE Immunoassay System (Ciba Corning) the result of 8690 RLU obtained in the patient's sample corresponded to 19 SU/ml and allergen class 2, the control values of at most 700 RLU are equivalent to class 0.

Immunoblot

To verify the patient's IgE reactivity to *Plasmopara viticola* and to characterize the putative allergens, we performed Western blot and IEF immunoblot analyses. The persons in groups 1, 2, and 3 served as control subjects. Using nitrocellulose as conventional blotting membrane, we were not successful in demonstrating clear-cut reactivities. This changed dramatically when we used CNBr-activated nitrocellulose. Then we were able to demonstrate in Western blot analysis (Fig. 2) one strong band at 21 kd and three faint bands at 50, 23, and 19 kd with the patient's IgE. We could not detect any IgE reactivity on the blot when probing sera control subjects. Moreover, when *Podosphaera leucotricha* was used as the control antigen to which the patient in question was exposed as well, we were not able to obtain any reactivities. We obtained the same result with the control subject. These findings were supported by the results of IEF immunoblot analysis. The patient's IgE was reactive to bands centered on isoelectric points 4.6 and 3.7, whereas IgE from the control subjects were negative.

FIG. 2. A, Western blot: IgE reactivity against *Plasmopara viticola* (1-5) and *Podosphaera leucotricha* (6-10); control 1 and 2 (6, 7); protein staining 3 (8); patient 4 (9); control sera 5 (10). **B**, IEF/immunoblot: IgE reactivity against *Plasmopara viticola* (1-7) and *Podosphaera leucotricha* (8-12); control 1 and 2 (8-9); protein staining 3 (10); patient 4 (11); control sera 5, 6, 7 (12).

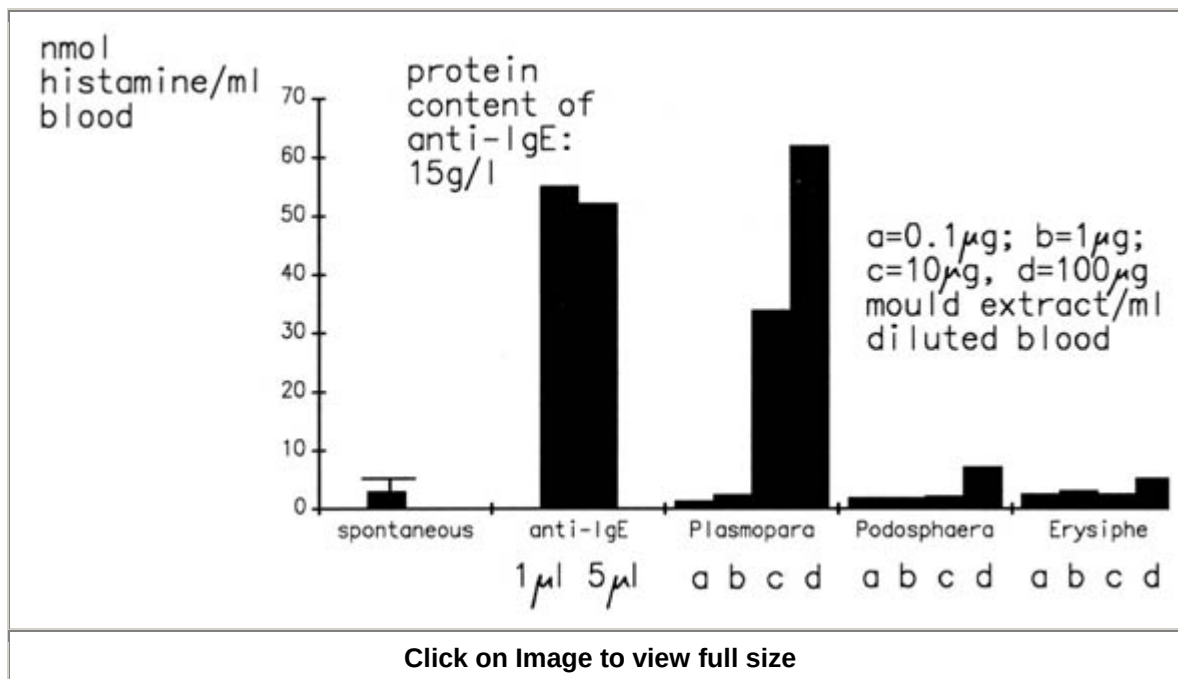




Histamine release test

The results of the histamine release test are summarized in Fig. 3. Only the blood of the 31-year-old greenhouse worker showed a significant histamine liberation induced by *Plasmopara viticola* at a minimal dose of 1 μ g extract per milliliter of blood. In the exposed coworker (control subject 1) no mold-induced histamine release could be observed up to 10 μ g extract per milliliter of blood. One hundred micrograms of extract of some molds was able to induce a slight histamine release in the patient, as well as in control subjects.

FIG. 3. Histamine release from diluted whole blood of the 31-year-old greenhouse worker by molds.



Bronchial provocation

The nonspecific provocation test with acetylcholine demonstrated severe hypersensitivity in the bronchial system of the patient. After inhalation of the second step doses (1.5 mg acetylcholine) the bronchial resistance increased from 0.17 to 0.87 kPa/L/sec in the patient. One of two control subjects in group 4 showed an increase from 0.19 to 0.69 kPa/L/sec after inhalation of the second-step doses (1.5 mg acetylcholine); the other showed an increase from 0.16 to 0.74 kPa/L/sec after inhalation of the fourth-step doses (15 mg acetylcholine).

The specific bronchial challenge test was performed with extract of *Plasmopara viticola*. After inhalation of cumulative 0.555 mg extract, the resistance of the patient increased by 67% within 30 minutes. This result does not fulfill the criterion for a positive challenge test result. Within 5 hours this value returned to baseline, dropping from 167% to 122% of the baseline value.

DISCUSSION

There are a number of molds that may cause type I, as well as type III, hypersensitivity.^{3, 5} The class of the *Oomycetes* has not been studied extensively by allergists until now.⁷ Only three cases of allergic respiratory symptoms to the *Oomycete* *Phytophthora infestans*, the false mildew of potatoes and cabbage, were described in 1969.²¹

The history of this patient suggested that there was a bronchial manifestation of an allergy to *Plasmopara*. The respiratory symptoms, such as rhinorrhoea and dyspnea, decreased when the patient was removed from his workplace for a few days. In the skin test the extract of *Plasmopara* caused an immediate positive response in a dose-dependent manner at a concentration of 0.5 µg/ml (7 mm wheal) and at 5 µg/ml (12 mm wheal). There was a significant difference between the minimal irritative reaction in control

subjects (wheal < 2 mm) and the positive reaction of the patient.

In the case of this greenhouse worker, IgE antibodies specific to *Plasmopara viticola* were clearly detected by a quantitative chemiluminescence immunoassay. The definitive involvement of IgE was confirmed by means of immunoblot technique. The major allergen has an apparent molecular weight of 21 kd; additional proteins bands appeared with molecular weights of 50, 23, and 19 kd. The studies with IEF immunoblot demonstrated that the allergens are acid substances with bands at isoelectric points 4.6 and 3.7. A positive histamine release test result with the extract demonstrates its intact biologic allergenic activity.

To verify a *Plasmopara*-induced bronchial obstruction we challenged the patient with bronchial provocation tests. The cumulative dose of inhaled allergen was limited to 0.555 mg to avoid endangering the patient. The induced obstructive reactions with an increase in bronchial resistance to 167% of the baseline value was not significant.²² We considered the repetition of this test with a higher dose to be unethical, because the patient has severe bronchial hyperreactivity. In this bronchial provocation test with 0.555 mg extract, *Plasmopara* could not be clearly identified as the allergic trigger factor for asthma. However, an obstructive reaction at higher concentrations of the allergen cannot be excluded. On the other hand, a sensitization to mold in the workplace was demonstrated only for *Plasmopara* and *Alternaria*. However, the patient is exposed frequently to *Plasmopara* and rarely to *Alternaria*. This patient does not handle the other molds, which caused a positive skin reaction like *Aspergillus* and *Cladosporium*. The work-related symptoms and the well-known exposure of the patient still suggest that *Plasmopara* is a relevant problem, but not the only one.

It was not our intention to determine the relative risk for sensitization from this investigation. However, the known general risks for sensitization must be considered: the atopic disposition and the special exposure enhance the probability of developing type I hypersensitivity.²³

Moreover, the special climatic conditions of the workplace were those of a greenhouse, high temperature combined with high humidity and poor air exchange.²⁴ It is not clear whether treatment of *Plasmopara* with fungicide has an effect on the induction of the allergy to this mold.

To our knowledge, this is the second time that a sensitization to a mold of the class of *Oomycetes* has been described.²¹ Whether this sensitization is a single case or one of epidemiologic significance must be demonstrated by further investigations.

REFERENCES

1. Lehrer SB, Lopez M, Butcher BT, Olson J, Reed M, Salvaggio JE. Basidiomycete mycelia and spore-allergen extracts: skin test reactivity in adults with symptoms of respiratory allergy. *J ALLERGY CLIN IMMUNOL* 1986;78:478-85.
2. O'Hollaren MT, Yunginger JW, Offord KP, et al. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* 1991;324:359-63.
3. Horner WE, Lopez M, Salvaggio JE, Lehrer SB. Basidiomycete allergy:

identification and characterization of an important allergen from *Calvatia cyathiformis*. Int Arch Allergy Appl Immunol 1991;94:359-61.

4. O'Neil CE, Horner WE, Reed MA, Lopez M, Lehrer SB. Evaluation of Basidiomycete and Deuteromycete (Fungi Imperfecti) extracts for shared allergenic determinants. Clin Exp Allergy 1990;20:533-8.
5. Salvaggio JE, Aukrust L. Mold induced asthma. Clin Immunol 1981;69:327-46.
6. Sridhara S, Gangal SV, Joshi AP. Immunochemical investigation of allergens from *Rhizopus nigricans*. Allergy 1990;45:577-86.
7. Giannini EH, Northey WT, Leathers CR. The allergenic significance of certain fungi rarely reported as allergens. Ann Allergy 1975;35:372-6.
8. Mendoza L, Kaufman L, Standard P. Antigenic relationship between the animal and human pathogen *Pythium insidiosum* and nonpathogenic pythium species. J Clin Microbiol 1987;25:2159-62.
9. Imwidthaya P, Srimuang S. Immunodiffusion test for diagnosing human pythiosis. Mycopathologia 1989;106:109-12.
10. Stangl A, Sattler W. Allergie-in-vitro-Diagnostik Chemilumineszenz-Detektion, magnetische Trennung und Allergen-Standardqualität. In-vitro Diagnostica Special 1990;1:28-34.
11. Haas H, Becker W-M, Maasch HJ, Schlaak M. Analysis of allergen components in grass pollen extracts using immunoblotting. Int Arch Allergy Appl Immunol 1986;79:434-40.
12. Demeulemester C, Peltre G, Laurent M, Panheleux D, David B. Cyanogen bromide activated nitrocellulose membranes: a new tool for immunoprint techniques. Electrophoresis 1987;8:71-3.
13. Kyhse-Andersen J. Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. J Biochem Biophys Methods 1984;10:203-9.
14. Neville DM, Glossmann H. Molecular weight determination of membrane protein and glycoprotein subunits by discontinuous gel electrophoresis in dodecyl sulfate. Methods Enzymol 1974;32:92-7.
15. Hancock K, Tsang VCW. India ink staining of proteins on nitrocellulose paper. Anal Biochem 1983;133:157-62.
16. Leary JJ, Brigati DJ, Ward DC. Rapid and sensitive colorimetric method for visualizing biotin-labeled DNA probes hybridized to DNA or RNA immobilized in nitrocellulose: bio-blots. Proc Natl Acad Sci U S A 1983;80:4045-9.
17. Schaubschläger WW, Becker W-M, Schade U, Zabel P, Schlaak M. Release of

mediators from human gastric mucosa and blood in adverse reactions to benzoate. *Int Arch Allergy Appl Immunol* 1991;96:97-101.

18. Schübeler K, Becker W-M, Schaubschläger WW, Schlaak M. Comparison of different radioimmunoassays (RIA) and radioenzymatic assay (REA) for the determination of histamine [Abstract]. *Allergy* 1988;43(suppl 7):71.

19. Schwaiblmair M, Baur X, Fruhmann G. Korrelation von Parametern Der fließvolumenkurve zum Ausmaß der bronchialen Hyperreagibilität. *Pneumologie* 1991;45:15-8.

20. Klein G, Wagner-Heck, Zinn CG. Inhalative carbachol provocation test using a reservoir method: 4-step test for bronchial hyperreactivity. *Pneumologie* 1991;45:679-83.

21. Liebeskind A. False-mildew (*Phytophthora infestans*) as an allergenic factor. *Acta Allergologica* 1969;24:362-6.

22. Subcommittee on 'Occupational Allergy' of the European Academy of Allergology and Clinical Immunology. Guidelines for the diagnosis of occupational asthma. *Clin Exp Allergy* 1992;22:103-8.

23. Cockcroft DW. Occupational asthma. *Ann Allergy* 1990;65:169-75.

24. Waegemaekers M, van Wageningen N, Brunekreef B, Boleij JSM. Respiratory symptoms in damp homes. *Allergy* 1989;44:192-8.

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