Case reports

A commentary on the following three case reports appears on pp 656–7.

Hypersensitivity pneumonitis induced by a smut fungus *Ustilago esculenta*

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Abstract

A case of hypersensitivity pneumonitis caused by a smut fungus *Ustilago esculenta* is presented.

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Keywords: hypersensitivity pneumonitis, smut, *Ustilago esculenta.*

Ustilago is the most prevalent smut fungus in grain.[1–4] It is listed as an aeroallergen associated with bronchial asthma, but not with hypersensitivity pneumonitis.[4–7] This is the first reported case of hypersensitivity pneumonitis to be caused by *Ustilago esculenta.* We present this case in order to raise the possibility of *Ustilago* induced hypersensitivity pneumonitis among grain workers and farmers.

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Case report

A 40 year old woman engaged in Japanese traditional handicrafts developed increasing dyspnoea, cough, and fever. She recovered completely after two weeks away from work but developed the same symptoms again at midnight on the day she returned to work.

The results of physical examination, routine laboratory studies, and pulmonary function tests were normal. A chest radiograph and high resolution computed tomographic scan showed diffuse bilateral fine nodular shadows without hilar adenopathy. Cultures for microorganisms including mycobacteria from sputum, bronchoalveolar lavage fluid, and gastric juice showed normal flora or were negative. The bronchoalveolar lavage was performed a week after the last exposure by methods described previously.[7] Of the 150 ml saline injected, 100 ml of bronchoalveolar fluid was recovered. The total cell yield was $78{\cdot}4 \times 10^6$ ($87{\cdot}1 \times 10^4$/ml), and the differential cell count was 54·7% lymphocytes, 41·5% pulmonary alveolar macrophages, 3·5% polymorphonuclear leucocytes, and 0·3% eosinophils with a CD4/CD8 ratio of 1·61. A transbronchial lung biopsy specimen revealed granulomatous alveolitis.

The causative antigen suspected was smut spores in her work place. She sprinkled these spores on lacquered wares and blew the excess off, producing a rusty colour. Macroscopically, the spores were brown and powdery, and microscopically they were single, globular or ellipsoid, measuring $5{\cdot}0–10{\cdot}2 \times 4{\cdot}2–6{\cdot}5$ μm. The smut teliospores were identified by morphometric examination as *Ustilago esculenta.*

Several procedures were conducted to prove that the spores of *U esculenta* were the causative antigen.

INDIRECT FLUORESCENT ANTIBODY (IFA) TEST

The method of Vogel, slightly modified, was performed as previously reported.[8,9] Spores of *U esculenta* and dust from the work place of the patient were tested with the patient's serum and with control serum samples. The spores were only reactive to the patient's serum among the dusts tested and showed an IFA titre of 1:512, while control serum samples showed less than 1:8.

GEL DOUBLE DIFFUSION TEST

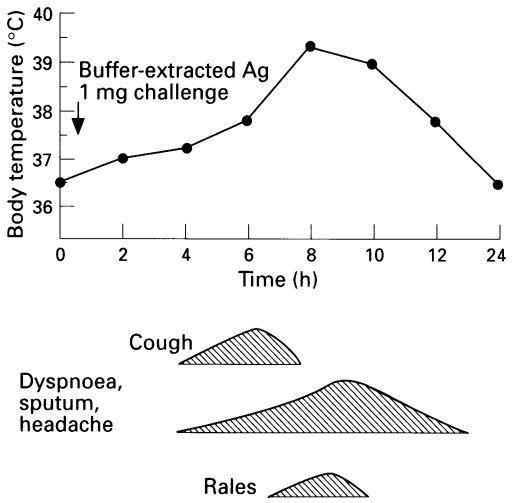
The method of Gerber and Jones, slightly modified, was performed as previously reported.[8,9] Antigen was extracted from the teliospores of *U esculenta* with sodium bicarbonate buffered saline by a modified version of Santilli's method.[6] The serum of the patient showed dense precipitins against the extracted antigen (10 mg dry weight/ml) and a faint precipitin against only *Aspergillus niger* from among common hypersensitivity pneumonitis related and *Aspergillus* related antigens (Hollister-Steir, Spokane, Washington).

LYMPHOCYTE PROLIFERATIVE RESPONSE TEST FOR PERIPHERAL BLOOD CELLS

Cellular incorporation of [^{3}H]thymidine was determined by the method of Moore and coworkers[9] and expressed as a stimulating index: the mean cpm of wells containing 1×10^5 lymphocytes (viability 98%) and antigen or mitogen divided by the mean cpm of wells without stimulant. Lymphoproliferative responses to the extracted antigen were observed in a dose dependent manner. The maximum net stimulation and stimulation index value were 5443 cpm and 23·8, respectively, at 0·8 μg/ml concentration. No significant response was observed in control cells.

SKIN TEST

Skin reaction to the extracted antigen was made by intradermal injection with 0·02 ml of a solution (0·1 mg/ml) and was read during the



WBC(μl)	6890	13 600	16 400
CRP (mg/dl)	0·25	0·25	7·41
Po_2 (kPa)	12·7	9·0	11·0
VC (l)	3·15	2·48	3·02

Results of inhalation challenge test with U esculenta performed on the patient using 1 mg of buffer extracted antigen inhaled through an ultrasonic nebuliser. The same provocation test did not show any effect on a healthy control subject.

Published reports of smut-related allergic respiratory diseases

Year	*Country*	*No of patients*	*Symptoms*	*Antigens*
1937	USA	1	Asthma	*Ustilago zeae* (=*maydis*), *Tilletia levis* (=*foetida*) etc
1939	USA	13	5 asthma, 6 hayfever, and 2 hayfever with asthma	*U tritici*, *U zeae*, *U avnae*, *T tritici*, *T levis*, etc.
1940	USA	103	Asthma	*T tritici*, *T levis*, *U zeae*
1940	USA	34	Asthma and/or hayfever	*Sphacelotheca sorghi*
1940	USA	19	17 asthma, 2 nasal allergy	*T tritici*, *T levis*, *U zeae* etc
1941	Spain	2	Asthma	*T caries*, *T foetida*
1952	Brazil	1	Asthma	*U tritici*
1957	France	5	Asthma	*U maydis*
1965	India	?	Respiratory troubles	*U virens*
1968	Japan	1	Asthma	*U esculenta?*
1979	Japan	2	Hypersensitivity pneumonitis	N.I.

NI = not identified. The antigen was not identified but the authors suspected *U esculenta* to be the causative antigen in these cases.
Details of the references are available from the authors on request.

next 48 hours. The patient showed immediate and Arthus-type reactions to the antigen but two healthy control subjects did not show any reaction.

INHALATION CHALLENGE TEST

Ten ml of the extracted antigen (0·1 mg/ml) were inhaled through an ultrasonic nebuliser (Omuron, Japan). Symptoms, signs, and laboratory findings were recorded during the next 48 hours. Symptoms and signs were reproduced five hours later with maximum effect nine hours later. Laboratory findings also worsened as shown in the figure. A healthy control subject who had an identical test did not show any abnormality.

The patient's symptoms have not recurred since moving to alternative employment.

Discussion

We have shown the smut fungus *U esculenta* to be an aetiological antigen of hypersensitivity pneumonitis. The patient reacted to the antigen and did not show any precipitins to common hypersensitivity pneumonitis related antigens tested. These results agree with findings utilising an animal model where spores of basidiomycetes (including smuts) possessed antigens which were not cross reactive with those of certain fungi imperfecti.[10]

U esculenta is distributed widely throughout Asia, and is parasitic on Manchurian wild rice (*Zizania latifolia*). In Japan the pure spores have been used as paint in the traditional lacquer industry. Hypersensitivity pneumonitis in millers who process wild rice that is infected by *U esculenta* has also been reported but the antigen was not defined as inhalation challenge was carried out using the whole flour (table).

Smuts were listed as provocative antigens in bronchial asthma.[4–7] Recently, Marx reported that the frequency of a positive skin test or RAST to grain smut was significantly higher (11·2%) among farming cases than controls (0%) in Wisconsin.[7]

At present, smuts are not as common in advanced countries as they used to be because of the development of fungicides and smut-resistant grains. However, *Ustilago* is still predominant in grain dust or among atmospheric spores.[1–3] Our observation of the dust from Saskatchewan grain elevators revealed that it still contained highly immunoreactive smut spores (unpublished data).

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