

Hypersensitivity Pneumonitis due to Bunashimeji Mushrooms in the Mushroom Industry

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Key Words

Bunashimeji mushrooms · Hypersensitivity pneumonitis · KL-6 · SP-D · Stimulation index

Abstract

Background: Detection of hypersensitivity pneumonitis (HP) in employees involved in the Bunashimeji mushroom industry is difficult. The level of precipitating antibody is not related with the prediction of progression and resolution of HP. The aims of this study were to examine the actual prevalence of HP in the Bunashimeji industry and the clinical differences among selected employees. **Methods:** One hundred and fourteen employees worked in Bunashimeji enterprises. These subjects were divided into the following subgroups: office workers, pickers/packers with mask and pickers/packers without mask. We measured serum Krebs von der Lungen-6 (KL-6), surfactant protein (SP)-A and SP-D, and examined the stimulation index (SI) due to Bunashimeji spores. Chest high-resolution computed tomography (HRCT), pulmonary function tests and bronchoalveolar lavage (BAL) were performed for select employees who showed positive SI values (>200%) to examine the clinical differences. **Results:** The proportion of respiratory symptoms was significantly higher in the pickers/packers than that in the office workers. The picker/packer group had high

serum KL-6 concentrations and SI compared with the office worker group. Thirty select employees were divided into the following three subgroups: HP, select employees without HP, and SI <400% and KL-6 <500 U/ml, using high SI levels (>400%) and positive serum KL-6 concentration (>500 U/ml). Four exhibited ground glass opacities with centrilobular fine nodules on HRCT, and 8 had high numbers of lymphocytes in the BAL fluid. The BAL findings and serum KL-6 concentrations showed significant differences among the three groups. **Conclusions:** Four employees were evaluated as having HP. Serum KL-6 and SP-D may be related to the resolution of HP in addition to SI and chest HRCT.

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Introduction

Hypsizygus marmoreus is called the 'Bunashimeji' mushroom in Japanese, and is commonly eaten fresh throughout Asia. In the Nagano prefecture, over 1,000 people are employed in the cultivation of Bunashimeji mushrooms. Some people who work in the Bunashimeji industry suffer from hypersensitivity pneumonitis (HP) due to chronic inhalation of Bunashimeji mushroom spores. We have previously reported the clinical features and diagnostic methods of HP induced by *Lyophyllum*

aggregatum spores [1]. Tanaka et al. [2] reported 2 cases of HP due to mushroom spores of *H. marmoreus*. The former is an old botanical name which is commonly known as the Bunashimeji mushroom. Its precise botanical name is *Hypsizygus marmoreus*, and the two mushrooms are equivalent.

In our previous study, all patients with HP had worked in the private enterprise for 10 years or longer. It was suggested that HP was caused by long-term inhalation of Bunashimeji spores, because patients had been employed in cultivating Bunashimeji mushrooms in an indoor environment. Bunashimeji spores were distributed throughout the occupational environment. However, we were not able to identify the occurrence of HP because of the mildness of the symptoms. Moreover, some cases had normal findings on their chest X-ray although chest CT revealed ground glass opacity and centrilobular fine nodules. Therefore, detection of HP in employees cultivating Bunashimeji mushrooms remains difficult, even if routine medical examinations including laboratory studies and chest X-rays have been performed. However, chest CT could detect ground glass opacity in cases of HP when the chest X-ray could not detect any abnormalities. In our previous study, the final diagnosis of Bunashimeji-mushroom-related HP required bronchoalveolar lavage (BAL) and transbronchial lung biopsy. To our knowledge, there is no reliable marker (clinical or laboratory) that predicts the progression or resolution of HP. The level of the precipitating antibody was not related to the prediction of progression and resolution of HP. Previous studies suggested that the immunopathological mechanisms for the development of HP involve both immune complex deposition and cell-mediated immunity [3]. The lymphocyte stimulation test (LST) is primarily regarded as a test of cell-mediated immunity (type IV) by lymphocytes [4]. One of the diagnostic aims for HP is that the LST can be used instead of precipitating antibody tests. Therefore, in Japan, positive results of the LST or precipitating antibody tests need to perform as a diagnostic criterion for HP.

It is impractical to perform detailed examinations on all employees to establish whether they suffer from HP. Therefore, we searched for methods using peripheral blood to predict employees who may have HP. The purpose of this study was to determine the existence of Bunashimeji-mushroom-related HP in employees working in the Bunashimeji industry, and to examine the clinical differences among selected employees.

Patients and Methods

Subjects

Our research protocol was approved by the Human Ethics Committee of the Shinshu University School of Medicine, including the use of invasive procedures. Prior to this study, we fully explained Bunashimeji-mushroom-related HP and the purpose and methods of the study to all employees who had worked at two Bunashimeji factories in the Nagano Prefecture. Signed informed consent was obtained from employees who were enrolled in the study. Blood samples were obtained from all participating employees. At first, we obtained information about the occupational category in the Bunashimeji industry from each employee, and medical information about whether they used a gauze mask, or had any of the following symptoms while working in the Bunashimeji industry: fever, cough, sputum, dyspnea or general fatigue. If workers responded affirmatively to one of these symptoms, they were asked to state the approximate period when they had first noticed the symptoms.

We classified the employees into three groups according to the quantity of exposure to Bunashimeji spores. Employees designated as the office group, i.e. office workers in the Bunashimeji industry facility, were not exposed to Bunashimeji spores and did not go to the pickers'/packers' room in the course of their work. They had contact with pickers/packers at the end of their workday. Employees designated as the picker/packer group were employed to pick up Bunashimeji mushrooms from the culture bottle and pack Bunashimeji mushrooms in boxes. Among the picker/packer group, employees who did not use masks were grouped as those without mask, and the others were grouped as with mask. The picker/packer group without masks did not wear masks out of their own personal choice. The picker/packer group with mask used a mask while working. These gauze masks were not designed to filter out respirable particles <5 µm. No employees had food allergies such as diarrhea after they ate Bunashimeji mushrooms. Chest X-rays were obtained within the past 1 year as a general health examination for all employees.

Twenty-two female general volunteers who had never been exposed to Bunashimeji spores, never smoked, and who had no atopic tendencies gave informed consent to participate in our study as normal controls. Blood samples were obtained from these volunteers and subjected to further examinations.

Study Design

After obtaining clinical information and conducting a physical examination, blood was sampled. We assessed complete blood cell counts, differential white blood cell counts, the erythrocyte sedimentation rate (ESR) and the LST [4] due to Bunashimeji spores using peripheral blood. The results of the LST were reported as the value of the stimulation index (SI), serum Krebs von der Lungen-6 [5] (KL-6; normal range: <500 units/ml), serum surfactant protein (SP)-A [6] (normal range; <43.8 ng/ml) and serum SP-D [7] (normal range: <110 ng/ml).

Diagnosis of HP

The criteria [1] used to establish the diagnosis of Bunashimeji-mushroom-related HP included the following: clinical features associated with ground glass opacity and small nodules on high-resolution CT (HRCT), predominance of lymphocytes in BAL fluid, alveolitis with infiltrating lymphocytes and histological granuloma formation in the alveoli, a positive precipitating assay

due to Bunashimeji spores and/or positive SI levels (>200%) due to Bunashimeji spores determined at the Special Reference Laboratory.

LST and Ouchterlony Method

Bunashimeji caps were placed in a case for several days, and spores were collected from the bottom of the case. The LST to Bunashimeji spores using peripheral mononuclear cells was performed as described previously [1] at the Special Reference Laboratory without providing any information about the clinical history. A total of 2×10^4 of Bunashimeji spores were added to each well containing 2×10^5 mononuclear cells/well. Bunashimeji spores were mixed vigorously with glass beads 0.4 mm in diameter (Iuchi, Osaka, Japan) for 30 s, and this mixing procedure was repeated 10 times to decompose the spores. Subsequently, these suspensions were centrifuged at 15,000 rpm for 10 min, and the supernatants were kept frozen at -70°C until use. To detect precipitating antibodies against Bunashimeji spore antigen, double immunodiffusion analysis (Ouchterlony) was performed [8].

Workers Selected for Further Examinations

After obtaining the results of blood samples and examining each finding, we determined the cutoff values for selecting subjects for the further study. The following cutoff value was used as a selection criterion: SI >200%. By mail, selected employees were approached to participate in further examinations.

Further Examinations

Chest CT and HRCT

We used a helical CT scanner (HiSpeed advantages; GE Medical Systems, Milwaukee, Wisc., USA) for the analysis. After standard 10-mm-thick contiguous scanning to screen chest abnormalities, scanning with 1-mm collimation was performed for the affected lesions. Two radiologists independently interpreted the findings of CT and high-resolution CT without information about the clinical history. The presence of centrilobular nodules measuring <5 mm, ground glass opacities and other findings were evaluated in each lobe.

Pulmonary Function Tests and Bronchoscopy

Pulmonary function tests (PFTs) were performed by our routine method [9]. Vital capacity (VC), forced expiratory volume in 1 s ($\text{FEV}_{1.0}$) and mean forced expiratory flow during the middle half of the forced VC ($\text{FEF}_{25-75\%}$) were measured by Chestac-65V (Chest, Tokyo, Japan). The functional residual capacity (FRC) using the helium dilution method and diffusing capacity for carbon monoxide (DL_{CO}) were measured by Chestac-55V (Chest). Residual volume was calculated from the FRC. The thoracic gas volume (TGV) was also measured by body plethysmography (model 1085/D; Medgraphics, St. Paul, Minn., USA). The percent VC, FEV_1 , FEV_1 percentage of predicted and DL_{CO} /alveolar volume were calculated. The peak expiratory flow rate and the flow rate at 25% of the forced VC (V'_{25}) were calculated from the maximum expiratory flow-volume curve. The percent VC, percent FEV_1 , FEV_1 percentage of predicted and DL_{CO} /alveolar volume were calculated according to the prediction equations of the Japanese Society of Chest Diseases.

BAL and transbronchial lung biopsies were performed following our laboratory methods [10]. The total cell counts of unfractionated BAL fluid were counted using a hemocytometer counting

chamber. Differential counts were performed using a smear of May-Giemsa stain. The pellet in the BAL fluid was analyzed for lymphocyte subsets by flow cytometry using the clusters of differentiation (CD) 4 and CD8 monoclonal antibodies (Becton Dickinson, Mountain View, Calif., USA). The specimens obtained by transbronchial lung biopsy were placed on glass slides, fixed with 10% formalin and processed for hematoxylin-eosin staining. All subjects were exposed to the antigen until the day before pulmonary function testing and bronchoscopy. Twenty-two female volunteers who served as control subjects had PFTs, and 7 of these control subjects underwent bronchoscopy.

Statistical Analysis

All values described in the text and tables represent mean $\pm$ SD. Student's unpaired t test, the χ^2 test for independence, and Spearman's rank correlation analysis were used for comparisons between the groups. A p value less than 0.05 was considered significant.

Results

Clinical Features

None of the 114 employees showed acute symptoms consistent with HP. Sixty-five employees (57.0%) complained of one or more symptoms. Their chief symptoms were chronic cough (66.2%), sputum (69.2%) and shortness of breath (4.6%). With regard to the clinical signs, 2 subjects showed emphysematous changes with increased infiltration, and 3 had mild fibrosis in the right and left lower lobes on chest X-ray. The median ages of the office workers, and the pickers/packers with mask and those without mask were 43.8, 55.8 and 56.2 years, respectively (range, 21–72 years).

As shown in table 1, we classified the 114 employees into three groups according to the extent of exposure to Bunashimeji spores. The proportion of respiratory symptoms was significantly higher in the pickers/packers than in the office workers ($p < 0.05$), but there was no significant difference between pickers/packers with mask and those without mask. Among the picker/packer group, 60 who did not use masks were grouped as without mask, and the others were grouped as with mask.

Laboratory Findings (table 1)

The SI due to Bunashimeji spores was positive in 86 of 114 employees (75.4%). The SI value was significantly higher in the picker/packer group than in the office worker and control groups. Among the picker/packer group, the SI of those with mask was higher than of those without mask. Among the 114 employees, serum KL-6 concentrations correlated significantly with serum SP-D concentrations ($r = 0.31$, $p < 0.05$), but not with SI or serum

Table 1. Characteristics of the employees and controls

	Control subjects (A)	Office workers (B)	Pickers/packers	
			with mask (C)	without mask (D)
Participants	22	21	33	60
Sex, males/females	0/22	8/13	1/32	12/48
Age, years	49.2 ± 7.0	43.8 ± 13.5	55.8 ± 8.8 ^d	56.2 ± 10.0 ^d
History of allergy	0	3	11	14
Rhinitis	0	1	7	7
Bronchial asthma	0	0	1	0
Pollinosis	0	1	1	5
Others	0	1	3	3
Smoking history				
Current smoker	0	4	0	4
Ex-smoker	0	0	0	0
Never-smoker	22	17	33	56
Duration of working, years	0	3.8 ± 1.8	4.8 ± 1.6 ^c	5.0 ± 2.3 ^c
Period until symptom onset, years	0	3.4 ± 2.1	4.1 ± 1.3	4.3 ± 2.1
Patients with symptoms	–	8 (38%)	24 (73%) ^c	33 (55%) ^c
Cough	0	7	17	19
Sputum	0	6	14	24
Shortness of breath	0	0	3	0
Laboratory data				
WBC/μl	6,030 ± 1,080	6,210 ± 1,300	5,700 ± 1,200	5,950 ± 1,500
ESR, mm/h	16 ± 8	14 ± 15	17 ± 9	18 ± 11
SI, %	198 ± 66	251 ± 115	454 ± 223 ^{b,d}	320 ± 184 ^{b,c,e}
KL-6, U/ml	251 ± 68	287 ± 83	339 ± 156 ^b	345 ± 208 ^{b,c}
SP-D, pg/ml	56.3 ± 24	77.3 ± 49	65.8 ± 44	81.5 ± 69 ^a
SP-A, pg/ml	31.2 ± 9.2	31.1 ± 13	34.2 ± 16	37.8 ± 17 ^a

Data are means ± SD. WBC = White blood cell count; ESR = erythrocyte sedimentation rate.

^a p < 0.05, ^b p < 0.001, vs. A; ^c p < 0.05, ^d p < 0.001, vs. B; ^e p < 0.05 vs. C.

SP-A concentrations. Serum SP-D concentrations correlated significantly with those of serum SP-A ($r = 0.21$, $p < 0.05$). There was no correlation between SI and serum KL-6 and SP-D concentrations. Serum KL-6 concentrations in the picker/packer groups were higher than those in the office worker and control groups, and there were significant differences between the pickers/packers without mask and office workers and controls.

Further Study

Eighty-six employees with a positive SI (>200%) were selected for this study. Thirty of the 86 employees (35%) participated in further examinations such as chest HRCT, PFTs and bronchoscopy. Fifty-six of the 86 employees did not elect to undergo further study. We regarded these 30 employees as the select employees. Thirteen of the 30 select employees wore the mask while working.

Serum KL-6, SP-D and SP-A concentrations and SI showed significant differences between select employees and control groups. Thirty select employees underwent chest HRCT. Four of 30 select employees showed ground glass opacity with centrilobular nodules and 3 of 30 select employees showed only centrilobular nodules on chest HRCT. Twenty-three select employees and 7 control subjects also had normal findings on chest HRCT.

Thirty select employees underwent PFTs. Two select employees showed an obstructive pattern on spirometry and had decreased DL_{CO}. FEV₁ and percent residual volume were not significantly different between the select employee group and the controls. Significant differences in V'25/height, FEF_{25–75%} and TGV-FRC between the controls and select employees revealed the existence of small airway diseases. Serum KL-6 concentrations correlated significantly with the values of percent VC as well

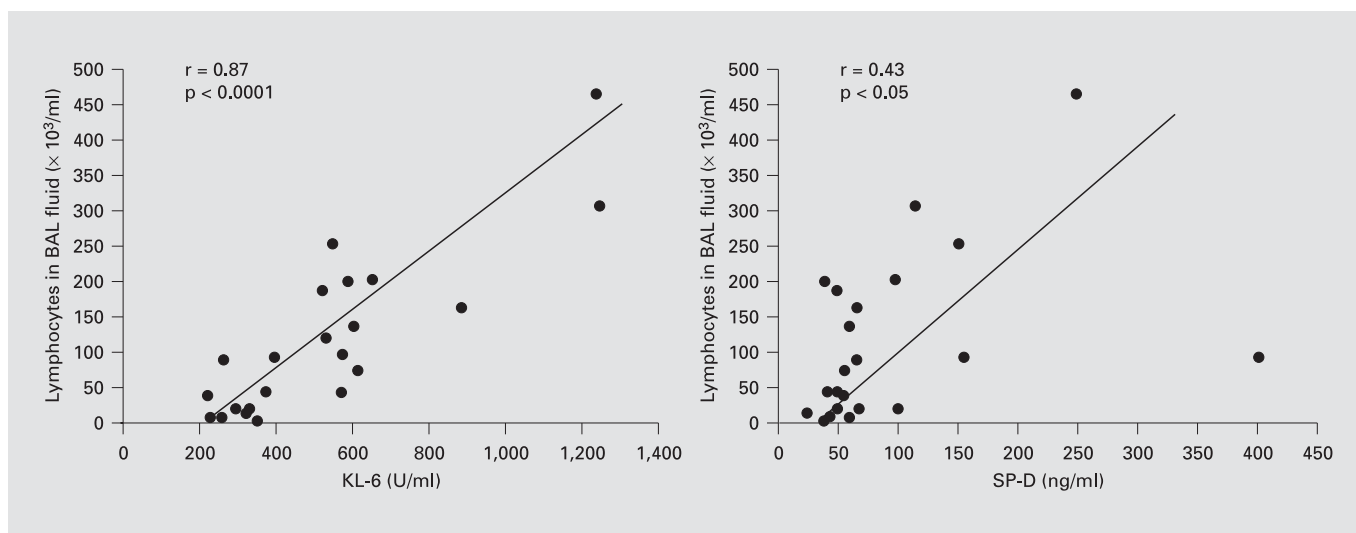


Fig. 1. Correlation between lymphocyte counts in BAL fluid and serum KL-6 or serum SP-D concentrations. Serum KL-6 concentrations ($r = 0.87$; $p < 0.0001$) and serum SP-D concentrations correlated significantly with lymphocyte counts in BAL fluid ($r = 0.43$; $p < 0.05$) in selected employees.

as with percent DL_{CO} ($r = 0.43$, $p < 0.05$; $r = 0.43$, $p < 0.05$, respectively). Serum SP-A concentrations correlated significantly with V'_{25}/height as well as with $FEF_{25-75\%}$ ($r = 0.43$, $p < 0.05$; $r = 0.44$, $p < 0.05$, respectively).

Thirty select employees underwent BAL and transbronchial lung biopsy. The percentages of lymphocytes, macrophages and neutrophils in BAL fluid were significantly different between the select employee group and the controls. In 8 of the select employee group, the number of lymphocytes in BAL fluid was high, and in 4 of these subjects the CD4/CD8 ratio was less than 1.0 in BAL fluid. Two of the 4 subjects worked without mask, and 4 subjects showed ground glass opacity with centrilobular nodules on chest HRCT. Three of 4 other select employees showed centrilobular nodules on chest HRCT. Serum KL-6 levels correlated significantly with the total cell counts and lymphocyte numbers in BAL fluid ($r = 0.82$, $p < 0.0001$; $r = 0.87$, $p < 0.0001$, respectively) (fig. 1). Serum SP-D concentrations also correlated significantly with total cell counts and lymphocyte numbers ($r = 0.58$, $p < 0.01$; $r = 0.43$, $p < 0.05$, respectively) (fig. 1). The SI and serum SP-A levels did not correlate with total cell counts and lymphocyte numbers in BAL fluid. The specimens obtained by transbronchial lung biopsy did not evidence small airway abnormalities histopathologically.

Diagnosis of HP

Four of 30 select employees were ultimately diagnosed as having HP caused by Bunashimeji spores, and 2 of them belonged to the group without mask ($n = 60$). All 4 employees diagnosed with HP complained of cough and sputum. They had high SI and serum KL-6 concentrations. Three of the 4 subjects had high serum concentrations of SP-D, and only 1 had a high serum SP-A concentration. The chest HRCT findings revealed mild ground glass opacity and centrilobular small nodules. The BAL findings included lymphocytosis and a low CD4/CD8 ratio, and specimens obtained by transbronchial biopsy revealed alveolitis with infiltrating lymphocytes and histological granuloma formation in the alveoli. Four subjects were shown to be positive in a precipitating assay according to the method of Ouchterlony.

Discussion

As reported previously [1], HP caused by Bunashimeji spores has conspicuous characteristics, e.g. mild respiratory symptoms, and in some cases, chest X-ray findings are normal. It is therefore difficult to detect HP by routine health checkups. HP is an insidious disease, with many years often passing before the onset of serious respiratory symptoms occur due to long-term inhalation of

Table 2. Comparison among select employees and controls

Group	Control subjects (A)	Select employees		
		SI <400 and KL-6 <500 (B)	select employees without HP (C)	HP (D)
Mask/without mask	0/22	4/3	7/12	2/2
SI, %	198 ± 66	306 ± 55 ^b	547 ± 191 ^{a,c}	513 ± 72 ^{a,d}
KL-6, U/ml	251 ± 68	271 ± 54	373 ± 124 ^{b,d}	981 ± 331 ^{b,d,e}
SP-D, ng/ml	56.3 ± 24	37.8 ± 11	92.8 ± 104 ^{a,c}	146 ± 78 ^{a,c}
SP-A, ng/ml	31.2 ± 9.2	39.8 ± 18	35.6 ± 8.3	34.6 ± 9.8
PFTs				
Subjects	22	7	19	4
VC, %	121 ± 8.9	111 ± 10	112 ± 8.5 ^b	102 ± 10 ^a
FEV ₁ , %	83.4 ± 5.6	80.2 ± 3.1	80.1 ± 10	84.5 ± 9.6
FEV ₁ /FVC, %	123 ± 16	106 ± 6.7	109 ± 5.1	91.6 ± 11
V'25/height	0.84 ± 0.31	0.56 ± 0.24 ^b	0.51 ± 0.23 ^b	0.58 ± 0.22
FEF _{25–75} , %	111 ± 30	90.9 ± 29	81.1 ± 27 ^b	88.4 ± 27
DL _{CO} , %	111 ± 8.5	96.5 ± 11 ^b	96.3 ± 9.2 ^a	84.2 ± 15 ^a
DL _{CO} /alveolar volume	5.7 ± 1.3	6.3 ± 0.67	5.9 ± 0.3	5.8 ± 1.8
Residual volume, %	123 ± 24	129 ± 58	154 ± 28 ^b	134 ± 26
TGV-FRC	0.20 ± 0.20	0.57 ± 0.30 ^b	0.68 ± 0.28 ^a	0.60 ± 0.35 ^b
BAL				
Subjects	7	7	19	4
TCC, × 10 ⁴ /ml	8.9 ± 4.9	6.0 ± 2.6	12.4 ± 9.0 ^c	43.5 ± 27 ^{a,c,f}
Lymphocytes, %	6.7 ± 3.1	20.4 ± 14	45.0 ± 19 ^{a,c}	72.5 ± 12 ^{a,c,f}
Macrophages, %	92 ± 4	73.9 ± 18	49.6 ± 18 ^{a,c}	25.5 ± 10 ^{a,c,f}
Neutrophils, %	1 ± 1	4.8 ± 3.7	4.9 ± 4.8	2.0 ± 2.2
CD4/CD8	1.80 ± 0.8	2.42 ± 1.5	2.1 ± 1.4	0.71 ± 0.5 ^{b,e}

Data are means ± SD. TCC = Total cell count.

^a p < 0.01, ^b p < 0.05, vs. A; ^c p < 0.01, ^d p < 0.05, vs. B; ^e p < 0.05, ^f p < 0.01, vs. C.

small amounts of spores. It must be differentiated from toxic alveolitis or the organic dust toxic syndrome. These reactions typically occur after a single exposure to an unusually high level of organic dust, and they occur without previous exposure [12]. The toxic alveolitis or organic dust toxic syndrome frequently results in neutrophilia, which may account for 20–50% of the cell differential count in BAL, but this neutrophilia was not observed in any of the groups studied. Their individual susceptibility is a characteristic feature of an immune-mediated disease, i.e. only a small percentage of those exposed to the antigen develop the disease. Therefore, we ruled out that the symptoms in their subjects were not due to organic toxic dust syndrome.

Important problems encountered in detecting HP include how to select employees who have HP, and how to select employees for further examination, e.g. HRCT,

PFTs or bronchoscopy. The mushroom factory consisted of several completely separated rooms: an office room, a room to pick and pack Bunashimeji mushrooms, and a room to cultivate Bunashimeji mushrooms. Employees were engaged in the same type of occupation throughout the year. Tanaka et al. [13] reported that the airborne endotoxin levels in the picking and harvesting rooms in the Bunashimeji industry were similar to those in other workplaces. We did not examine the airborne endotoxin levels in each room. We first separated employees into two groups based on the expected level of exposure to Bunashimeji spores; the picker/packer group in whom subjects were expected to be heavily exposed to spores; these subjects reported significantly more complaints in comparison with the control subjects and the office workers. The SI value enabled the identification of those employees who were engaged in the Bunashimeji industry and

had been exposed to Bunashimeji spores. Employees showing HP inhaled continuously a fixed small quantity of Bunashimeji spores, and these subjects revealed a high SI value. As shown in table 1, since the SI values of the office workers and controls were significantly lower than those of the picker/packer group, the former two groups may have been exposed to no or very low levels of Bunashimeji spores. They are not sensitized to the spores. Therefore, the SI can be used to discriminate patients with the disease from those without. In general, SI values are regarded as positive within 200% according to the Special Reference Laboratory. In the present study, when a cutoff value of SI >200% was selected to divide the employees, 75.4% of the employees were considered to have a positive SI. In our previous study [1], the lowest SI of subjects with HP due to Bunashimeji mushroom was 409%. In the present study, the SI values >400% were higher than the mean value of the unexposed employees, and employees diagnosed with HP showed >500%. Only 9% of the office worker group had an SI of this value. All control subjects had an SI value <400%. The median SI value was 313% in 49 employees without complaints in the Bunashimeji industry; 40 of 49 employees (82%) had an SI <400%. SI values >400% may be useful to select patients with increased sensitivity to Bunashimeji spores requiring further examinations. The differences in the SI levels are due to the intensity and duration of exposure (data not shown). Therefore, the usefulness and clinical interpretation of a positive SI is a mere marker of exposure.

Serum KL-6, SP-A, and SP-D concentrations are good markers of disease activity in interstitial pneumonitis such as idiopathic pulmonary fibrosis [4, 14–16]. The KL-6 level in BAL fluid was elevated in patients with idiopathic pulmonary fibrosis and correlated with the number of lymphocytes and total cell counts in BAL fluid [17]. Increased numbers of lymphocytes in BAL fluid reflect alveolitis activity and correlated well with interstitial involvement observed on chest CT in HP [18, 19]. A number of studies suggested that serum SP-D concentration was a good marker of disease activity in mushroom workers with HP, and that in patients with HP serum SP-A was moderately increased compared with controls [20]. In the present study, the sensitivity and specificity of serum KL-6 for identifying HP in 30 select employees were 100 and 88.5%, respectively.

Based on the above discussions, we classified 30 select employees into three groups: 4 patients with HP, 19 select employees without HP and 7 employees having SI <400% and KL-6 <500 U/ml (table 2). The SI differed signifi-

cantly between the controls and the three other groups. Serum KL-6 and SP-D concentrations were within the normal ranges in the SI <400% and KL-6 <500 U/ml group. The SI, serum KL-6, SP-D, and lymphocyte predominance in total cell counts in BAL fluid were significantly different between the SI <400% and KL-6 <500 U/ml group and the select employees without HP and the HP groups. Four HP patients had a significantly increased total cell count with lymphocyte predominance and high serum KL-6 concentrations. Three of the select employees without HP had high serum KL-6 and/or SP-D concentrations, but serum SP-A concentrations were within the normal range. They had high total cell counts with lymphocyte predominance in the BAL fluid. Two employees with SI <400% and KL-6 <500 U/ml and increased serum SP-A concentrations had normal PFTs and no abnormal findings in BAL fluid. With regard to BAL fluid findings, there were no significant differences between the control and the SI <400% and KL-6 <500 U/ml groups. Therefore, individuals of the SI <400% and KL-6 <500 U/ml group who served as controls for the select employees were regarded as subjects with a low likelihood of suffering from HP. Tanaka et al. [13] reported other respiratory conditions affecting Bunashimeji employees may cause clinical symptoms similar to those described in our study. The data of the remaining employees must be carefully interpreted.

In conclusion, at least 4 of 114 employees were evaluated as having HP. Based on the results among the three groups and selected employees, laboratory data, chest HRCT findings and BAL fluid findings showed significant differences according to the extent of spore exposure according to the SI value. Serum KL-6 and SP-D concentrations may be related to the resolution of HP.

References

- ▶ 1 Tsushima K, Fujimoto K, Yamazaki Y, Takamizawa A, Amari T, Koizumi T, Kubo K: Hypersensitivity pneumonitis induced by spores of *Lyophyllum aggregatum*. *Chest* 2001;120:1085–1093.
- ▶ 2 Tanaka H, Sugawara H, Saikai T, Tsunematsu K, Takahashi H, Abe S: Mushroom worker's lung caused by spores of *Hypsizygus marmoreus* (Bunashimeji): Elevated serum surfactant protein D levels. *Chest* 2000;118:1506–1509.
- ▶ 3 Yoshizawa Y, Nakazawa T, Ripani LM, Moore VL: Development of chronic pulmonary inflammation in immunized guinea pigs by aerosol challenge with antigen: Relationship of immune complex disease and cell-mediated hypersensitivity. *J Allergy Clin Immunol* 1982;70:114–119.
- ▶ 4 Hirshhorn K, Bach F, Kolodny RL, Firschein IC, Hashem N: Immune response and mitosis of human peripheral blood lymphocytes in vitro. *Science* 1963;142:1185–1187.
- ▶ 5 Kohno N, Kyoizumi S, Awaya Y, Fukuhara H, Yamakido M, Akiyama M: New serum indicator of interstitial pneumonitis activity: Sialylated carbohydrate antigen KL-6. *Chest* 1989;96:68–73.
- ▶ 6 Kuroki Y, Fukuda Y, Takahashi H, Akino T: Monoclonal antibodies against human pulmonary surfactant apoproteins: Specificity and application in immunoassay. *Biochim Biophys Acta* 1985;836:201–209.
- ▶ 7 Inoue T, Matsuura E, Nagata A, Ogasawara Y, Hattori A, Kuroki Y, Fujimoto S, Akino T: Enzyme-linked immunosorbent assay for human pulmonary surfactant D. *J Immunol Methods* 1994;173:157–164.
- ▶ 8 Holland P, Mauer AM: Drug-induced in-vitro stimulation of peripheral lymphocytes. *Lancet* 1964;i:1368.
- ▶ 9 Orjan Ouchterlony: Diffusion-in-gel methods for immunological analysis. *Progr Allergy* 1958;5:1–78.
- ▶ 10 Kubo K, Yamazaki Y, Masubuchi T, Takamizawa A, Yamamoto H, Koizumi T, Fujimoto K, Matsuzawa Y, Honda T, Hasegawa M, Sone S: Pulmonary infection with *Mycobacterium avium-intracellulare* leads to air trapping distal to the small airways. *Am J Respir Crit Care Med* 1998;27:979–984.
- ▶ 11 Kubo K, Hanaoka M, Hayano T, Miyahara T, Hachiya T, Hayasaka M, Koizumi T, Fujimoto K, Kobayashi T, Honda T: Inflammatory cytokines in BAL fluid and pulmonary hemodynamics in high-altitude pulmonary edema. *Respir Physiol* 1998;111:301–310.
- ▶ 12 Malmberg P, Rask-Anderson A, Rosenhall L: Exposure to microorganisms associated with allergic alveolitis and febrile reactions to mold dust in farmers. *Chest* 1993;103:1202–1209.
- ▶ 13 Tanaka H, Saikai T, Sugawara H, Takeya I, Tsunematsu K, Matsuura A, Abe S: Workplace-related chronic cough on a mushroom farm. *Chest* 2002;122:1080–1085.
- ▶ 14 Kobayashi J, Kitamura S: KL-6: A serum marker for interstitial pneumonia. *Chest* 1995;108:311–315.
- ▶ 15 Kuroki Y, Tsutahara S, Shijubo N, Takahashi H, Shiratori M, Hattori A, Honda Y, Abe S, Akino T: Elevated levels of lung surfactant protein A in sera from patients with idiopathic pulmonary fibrosis and pulmonary alveolar proteinosis. *Am Rev Respir Dis* 1993;147:723–729.
- ▶ 16 Honda Y, Kuroki Y, Matsuura E, Nagae H, Takahashi H, Akino T, Abe S: Pulmonary surfactant protein D in sera and bronchoalveolar fluids. *Am J Respir Crit Care Med* 1995;152:1860–1866.
- ▶ 17 Kohno N, Awaya Y, Oyama T, Yamakido M, Akiyama M, Inoue Y, Yokoyama A, Hamada H, Fujioka S, Hiwada K: KL-6, a mucin-like glycoprotein, in bronchoalveolar lavage fluid from patients with interstitial lung disease. *Am Rev Respir Dis* 1993;148:637–642.
- ▶ 18 Trentin L, Migone N, Zambello R, di Celle PF, Aina F, Feruglio C, Bulian T, Masciarelli M, Agostini C, Cipriani A: Mechanisms accounting for lymphocytic alveolitis in hypersensitivity pneumonitis. *J Immunol* 1990;145:2147–2154.
- ▶ 19 Engeler CE, Tashjian JH, Trenkner SW, Walsh JW: Ground-glass opacity of the lung parenchyma: A guide to analysis with high-resolution CT. *AJR Am J Roentgenol* 1993;160:249–251.
- ▶ 20 Takahashi H, Fujishima T, Koba H, Murakami S, Kurokawa K, Shibuya Y, Shiratori M, Kuroki Y, Abe S: Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. *Am J Respir Crit Care Med* 2000;162:1109–1114.