

## ASPERGILLUS CANDIDUS: A RESPIRATORY HAZARD ASSOCIATED WITH GRAIN DUST

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**Abstract:** The concentration of *Aspergillus candidus* in samples of grain dust and of air polluted with grain dust was found to be large (respectively  $3.0 \times 10^5$  -  $3.0 \times 10^9$  cfu/g and  $5.0 \times 10^3$  -  $6.47 \times 10^5$  cfu/m<sup>3</sup>) and proved to be significantly greater compared to samples of other organic dusts ( $p < 0.001$ ). Rabbits exposed to long-term inhalation of the cell extract of *A. candidus* revealed a positive cellular and humoral response, demonstrated by the significant ( $p < 0.01$ ) inhibition of leukocyte migration in the presence of specific antigen and by production of precipitins against antigen of the fungus. The inhibition of leukocyte migration was even stronger in another group of rabbits exposed twice to the inhalation of live *A. candidus* spores. A group of grain workers reacted significantly more frequently to extract of *A. candidus* in the leukocyte migration inhibition test ( $p < 0.01$ ) and precipitation test ( $p < 0.05$ ), compared to the control group not exposed to organic dusts. It was concluded that *Aspergillus candidus*, because of its common occurrence and strong immunomodulating properties, poses an important occupational hazard for grain handling workers.

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### INTRODUCTION

Filamentous fungi developing on plant matter present a major respiratory hazard for workers exposed to the inhalation of organic dusts. The substances released in the lungs from inhaled spores and fragments of mycelium may cause allergic diseases (allergic alveolitis, asthma) and/or immunotoxic diseases (organic dust toxic syndrome, mycotoxicoses, building-related disease) [7, 18, 25, 36, 37]. The adverse immunotoxic reactions may be elicited by (1→3)-β-D-glucans, mycotoxins, volatile organic compounds (VOCs) and enzymes [14, 25, 35, 36, 37, 40, 50].

Fungi of the genus *Aspergillus* were proved to be a common cause of diseases related to the exposure to organic dusts [12, 23, 24, 37]. Their potential inflammatory properties were confirmed by experiments

in which non-specific activation of complement and immunocompetent cells was found in animals exposed to the inhalation of *Aspergillus* spores [3, 6, 8, 31, 32, 47]. Primary pathogenic role has been attributed to such species as: *A. fumigatus*, *A. terreus*, *A. rubrobrunneus* (synonym: *Aspergillus umbrosus*, the anamorph of *Eurotium rubrum*), *A. niger*, *A. clavatus*, *A. flavus* and *A. versicolor* [1, 8, 18, 23, 31, 37, 45]. Much less is known about the role played by other *Aspergillus* species, including *Aspergillus candidus* reported by some authors as a common contaminant of organic dusts [16, 22, 33, 45].

*Aspergillus candidus* possesses white, typically globose, conidial heads producing globose or subglobose, smooth, thin-walled conidia measuring 2.5–3.5 μm in diameter. The fungus is widely distributed in nature and develops upon vegetation in the later stages of decay [34].

It has been reported from grain, flour, hay, compost and a fur processing facility [15, 16, 17, 18, 21, 22, 27, 33, 45]. According to Lacey [22] the optimal conditions for growth of *A. candidus* in barley grain occur at the substrate water content 20–25% and maximal temperature 30–40°C. In favourable conditions *A. candidus* may produce citrinin and other mycotoxins [50]. Its possible role in human occupational pathology has been suspected by our group since early 1980s when we found that the collective occurrence of organic dust toxic syndrome (ODTS) cases (originally diagnosed as an acute allergic alveolitis) among students employed at shuffling mouldy grain in the Szczecin region (north-western Poland) was associated with an abundant contamination of grain and grain dust with *A. candidus* [11, 43]. This species has also been found in samples of organic dusts associated with outbreaks of ODTS in the USA [41, 42, 49].

The aim of the present work was an attempt to evaluate the role of *Aspergillus candidus* as a potential respiratory pathogen for people exposed to organic dusts. The evaluation has been carried out on the basis of aerobiological and immunological studies, the latter including both experimental and epidemiological investigations. Preliminary results of this study have been reported elsewhere [19].

## MATERIALS AND METHODS

### Environmental studies

**Examination of the air samples.** Air samples were collected in following facilities located in central and eastern Poland (numbers of examined samples in parentheses), where plant materials were stored and processed: A - Grain elevator 1 in Lublin province: transporting belts (2); B - Grain elevator 2 in Rzeszów province: transporting belts (5); C - Grain mill in Rzeszów province: cleaning room (2); D - Animal feed production facility in Rzeszów province: milling wheat grain (2); E - Herb processing facility in Lublin province: grinding sage (5); F - Store of horticulture seeds in Warsaw province: sacking of the seeds of canary grass (*Phalaris canariensis*) (5); G - Tobacco processing plant in Lublin province: cutting tobacco leaves (5); H - Sawmill in Lublin province: frame cutting pine logs (5).

Air samples were collected on malt agar (*Difco*) plates with a custom-designed particle-sizing slit sampler, enabling estimations of the total and respirable fractions of the microbial aerosol [10]. The plates were subsequently incubated for 4 days at 30°C and 4 days at 22°C [29]. The grown colonies were counted and classified with microscopic methods, according to Barron [2], Litvinov [26], and Raper & Fennell [34]. The data were reported as cfu (colony forming units) of *A. candidus* per 1 cubic meter of air and as percent of *A. candidus* among total fungi.

**Examination of the samples of plant materials and settled dusts.** The following plant materials and dusts (numbers of investigated samples in parentheses), collected in various regions of Poland, were examined for the presence of *Aspergillus candidus*: A - grain dust from barley collected on a farm in Szczecin province, associated with ODTS cases among groups of students (1); B- grain dust from wheat collected in a mill in Lublin province (1); C - barley grain collected on a farm in Szczecin province, associated with ODTS cases among groups of students (1); D- rye grain collected on a farm in Szczecin province, associated with ODTS cases among groups of students (1); E - Dust from oak wood, collected in a sawmill in Lublin province (1); F - Seeds of the grass *Phalaris canariensis* collected in a store of horticulture seeds in Warsaw province (1); G - straw collected on the farm of a patient with diagnosis of allergic alveolitis in Radom province (1); H - Ground herbs, collected in herb processing plants in Białystok and Lublin provinces (5).

Samples were collected in the sterile Erlenmayer flasks. The concentration of total fungi and of *A. candidus* were determined by dilution plating. One gram of each sample was suspended in 100 ml of the sterile saline (0.85 % NaCl) containing 0.1% (v/v) of Tween 80, and after vigorous shaking, serial 10-fold dilutions in saline were made up to 10<sup>-10</sup>. The 0.1 ml aliquots of each dilution were spread on duplicate sets of malt agar (*Difco*) plates. The plates were incubated and examined as given above. The data were reported as cfu (colony forming units) of *A. candidus* per gram of the sample and as percent of *A. candidus* among total fungi.

**Examination of the isolated strains of *Aspergillus candidus*.** Six isolated strains of *A. candidus* were selected for examination by electron microscopy and for immunological studies. Four of them, marked AC-I, AC-II, AC-III and AC-IV, were isolated from samples of grain and grain dust associated with the ODTS outbreaks in Szczecin region: AC-I from rye grain, AC-II from rye dust, AC-III from barley dust, and AC-IV from barley grain. The strain AC-V of *A. candidus* was isolated from a sample of oats grain collected in the grain elevator in Lublin region, and the strain AC-VI of *A. candidus* was the reference strain obtained from the Central Laboratory for Storing and Processing Grain in Warsaw.

Ultrastructural examination of the spores of *A. candidus* isolates was carried out as described earlier [19, 29] in the Institute of Pediatrics of Jagiellonian University in Kraków. Briefly, small portions of conidial powder collected from 4-week malt agar slant cultures were prefixed in 2% glutaraldehyde in phosphate buffer at pH 7.3, and post-fixed in 1% buffered osmium tetroxide. After dehydration in graded series of ethanol, the samples were embedded in Low Viscosity (by dr Spurr), thin sectioned (silver colour) and post-stained with 2% uranyl acetate and lead citrate [29]. The micrographs were taken with a Philips EM 300 electron microscope operating at 80 KV.

### Experimental exposure of animals to the inhalation of *Aspergillus candidus* aerosol

Rabbits were exposed to the inhalation of *Aspergillus candidus* aerosol in two experiments (1 and 2). The cellular and humoral response of animals was assessed respectively by the test for the inhibition of leukocyte migration in the presence of specific antigen (MIF test) and by the agar-gel precipitation test. The tests were carried out with the circulatory blood samples taken at post exposure fixed intervals.

**Experiment 1.** Female rabbits of mixed race weighing 4.5-5.0 kg were divided into two groups, six animals each, for exposure to: a) extract of the *A. candidus* mycelium, prepared as described below (experimental group); b) sterile saline (0.85% NaCl) (control group). The extract of *A. candidus* mycelium was prepared as follows: cultures of the *A. candidus* AC-III strain incubated on liquid Sabouraud medium at 30°C for 4 weeks were homogenised and extracted in saline (0.85% NaCl) in the proportion 1:2 for 48 hrs at 4°C, with intermittent disruption of cells by 10-fold freezing and thawing. Afterwards, the supernatant was separated by centrifugation, dialysed against distilled water for 24 hrs, concentrated by evaporation to 0.15 of previous volume and lyophilised.

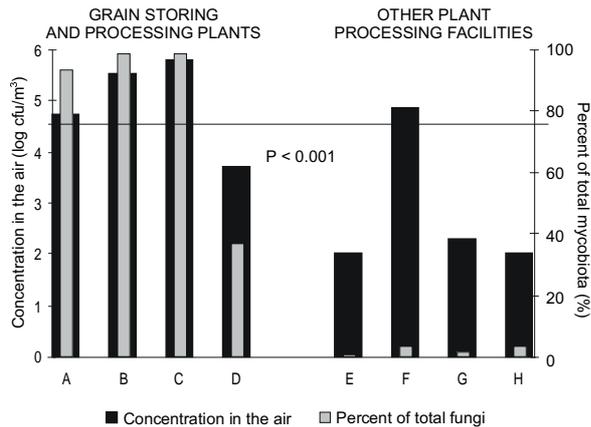
Rabbits belonging to each group were placed in an plexiglass inhalation chamber 150 × 100 × 80 cm, constructed according to the design of Kuš [20]. A fine aerosol was generated from the suspension of *A. candidus* lyophilisate in 0.85% NaCl, dissolved at the concentration of 5 mg/ml, sterilized by filtration, and fed into the chamber by the ultrasonic TUR-USI-3 nebulizer (produced in Germany). Animals were exposed 15 times, every second day for 1 hr, the experiment for each group (experimental and control) therefore lasted one month. Blood samples for MIF and precipitation tests were taken from the ear vein of each rabbit before experiment and after the 1st, 5th, 10th and 15th exposure (24 hrs after the 1st exposure and 4 hrs after further exposures). After the experiment, the rabbits were euthanased by injecting sodium pentobarbital and specimens of lung tissue were excised for histopathological examinations. The samples were fixed in 10% formaldehyde, embedded in paraffin, sectioned (7 µm) and dyed with H + E.

**Experiment 2.** Female rabbits of mixed race weighing 4.5-5.0 kg were divided into two groups, 6 animals each, for exposure to: a) spore dust of *A. candidus*, prepared as described below (experimental group); b) pulverized chalk, an immunologically inert substance having similar dispersion and consistence to spore powder (control group). A total of 20 gm of fine dust consisting of the spores of *A. candidus* (spore powder) was collected from 120 Sabouraud agar plates inoculated with the strain AC-III of *A. candidus* and incubated for 7 weeks at 30°C.

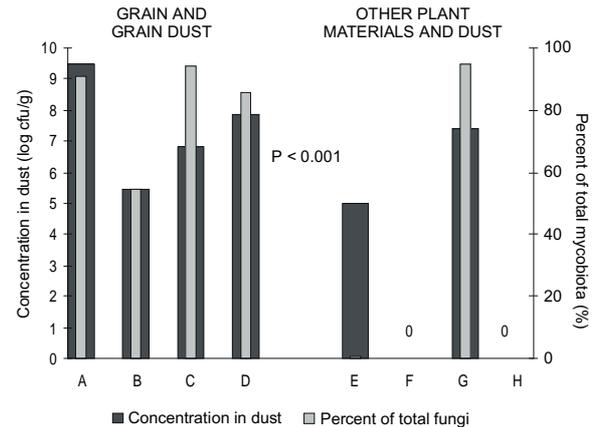
After collection to Erlenmayer flask, the spores were dried for 10 days at 40°C. It was found by dilution plating that 1 gm of spore powder consisted of  $1.5 \times 10^{10}$  live spores. Rabbits belonging to each group were placed in the inhalation chamber described above. An amount of 5 gm of spore powder was placed in a sack made from sterile gauze hung underneath the lid and the spores were dispersed into the air of the chamber within 15 minutes with the aid of a fan installed inside the chamber. Immediately afterwards, the entire operation was repeated, so that the total exposure time amounted to 30 minutes. The experiment was repeated after 6 days. It was estimated that the concentration of *A. candidus* spores in the air of inhalation chamber was about  $6.25 \times 10^{10}/m^3$ . The control rabbits were exposed twice to pulverized chalk, using the above technique. Blood samples for MIF and precipitation tests were taken from the ear vein of each rabbit from both groups before experiment and after the first and second exposure (24 hrs after the 1st exposure and 4 hrs after the 2nd exposure).

**Test for inhibition of leukocyte migration in the presence of specific antigen.** The test was performed by the whole blood capillary microculture method according to Bowszyc *et al.* [4]. Rabbit's blood and Parker's culture medium were added in the volumes of 0.5 ml and 0.12 ml, respectively, to 2 silicon test tubes. Then, 0.12 ml of the *A. candidus* antigen solution (prepared as for the above described extract for inhalation) in the concentration of 25 µg/ml was added to one tube, while to the other 0.12 ml of the diluent (P.B.S.) as a control. Both suspensions were incubated for 30 min at room temperature and thereafter distributed to heparinised glass capillars 75 × 1 mm. Capillars were sealed at both ends with the 4:1 mixture of paraffin and vaseline, centrifuged for 10 min at 1,500 rev/min and fastened tangentially on microscopic slides with sticky tape at an angle of 10°. The microcultures thus obtained were incubated for 4 hrs at 37°C in a humid chamber. The leukocyte migration distances, visible as distinct white zones, were measured under a binocular microscope. The results were expressed as a migration index (MI), e.g. the ratio of the mean migration distance of leukocytes in microcultures with antigen, to the analogical distance in microcultures without antigen. The test was considered as positive at an MI equal to 0.790 or lower.

**Agar-gel precipitation test.** The test was performed by Ouchterlony double diffusion method in purified 1.5% Difco agar. The rabbit's serum was placed in the central well and *A. candidus* antigen, at the concentration of 30 mg/ml, in the peripheral wells. Each serum was tested twice: not concentrated, and 3-fold concentrated, for the detection of low levels of precipitins. The plates were incubated for 6 days at room temperature, then washed in saline and in 5% sodium citrate solution (for preventing false positive reactions), and stained with azocarmine B [29].



**Figure 1.** Concentration of *Aspergillus candidus* spores in the air of various facilities, shown as decimal logarithms of cfu numbers per 1 m<sup>3</sup> (outer bars). Inner bars show percent of *A. candidus* in total fungal flora recovered from the air. Horizontal line indicates suggested occupational exposure limit value for airborne fungi. A - grain silo 1, B- grain silo 2, C - grain mill, D - animal feed production facility, E - herb processing plant, F - store of horticulture seeds, G - tobacco processing plant, H - sawmill. The concentration of *Aspergillus candidus* in the air samples from grain processing plants was significantly greater ( $p < 0.001$ ) than in the samples from other plant processing facilities.



**Figure 2.** Concentration of *Aspergillus candidus* spores in various plant materials and dusts, shown as decimal logarithms of cfu numbers per 1 gram (outer bars). Inner bars show percent of *A. candidus* in total fungal flora recovered from the dust. A - grain dust 1 (from barley), B- grain dust 2 (from wheat), C - barley grain (mill), D- rye grain, E - wood dust, F - grass seeds, G - straw, H - herbs. The concentration of *Aspergillus candidus* in the samples of grain and grain dust was significantly greater ( $p < 0.001$ ) than in the samples of other plant materials and dusts.

### Immunological study of exposed human population

**Examined population.** The study comprised 26 grain workers employed in an elevator in the city of Lublin, in which large quantities of *A. candidus* were detected during aerobiological studies. All the examined workers were males and their average age was  $37.2 \pm 9.8$  years. Most of the examined workers (21 persons) reported work-related symptoms after exposure to grain dust (dyspnea, cough, chest tightness, headache, burning eyes, rash, rhinitis). Eleven persons reported only one symptom while the remaining 10 persons reported two or more symptoms, including chronic cough and dyspnea.

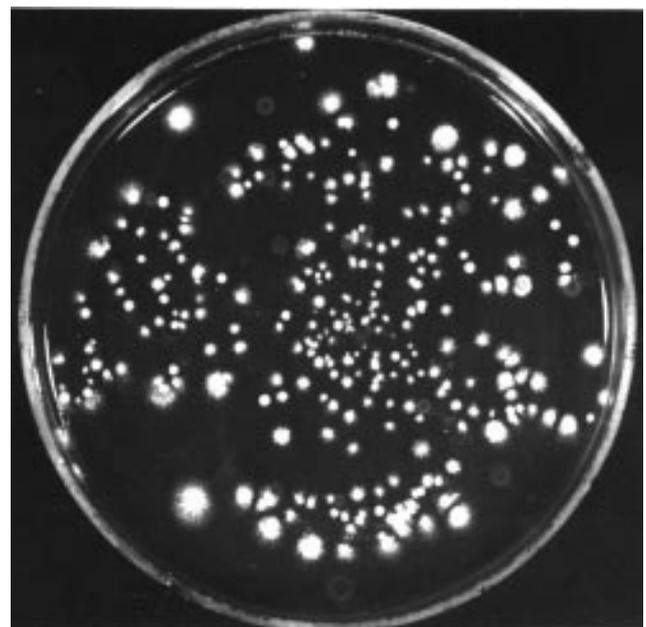
Thirty two healthy urban dwellers from the city of Lublin, without any contact with organic dust, were examined as a control group. The group consisted of 11 males and 21 females and their average age was  $36.4 \pm 8.6$  years.

**Tests.** Test for the inhibition of leukocyte migration (MIF test) in the presence of *A. candidus* antigen and the agar-gel precipitation test with *A. candidus* antigen were carried out, using techniques described above.

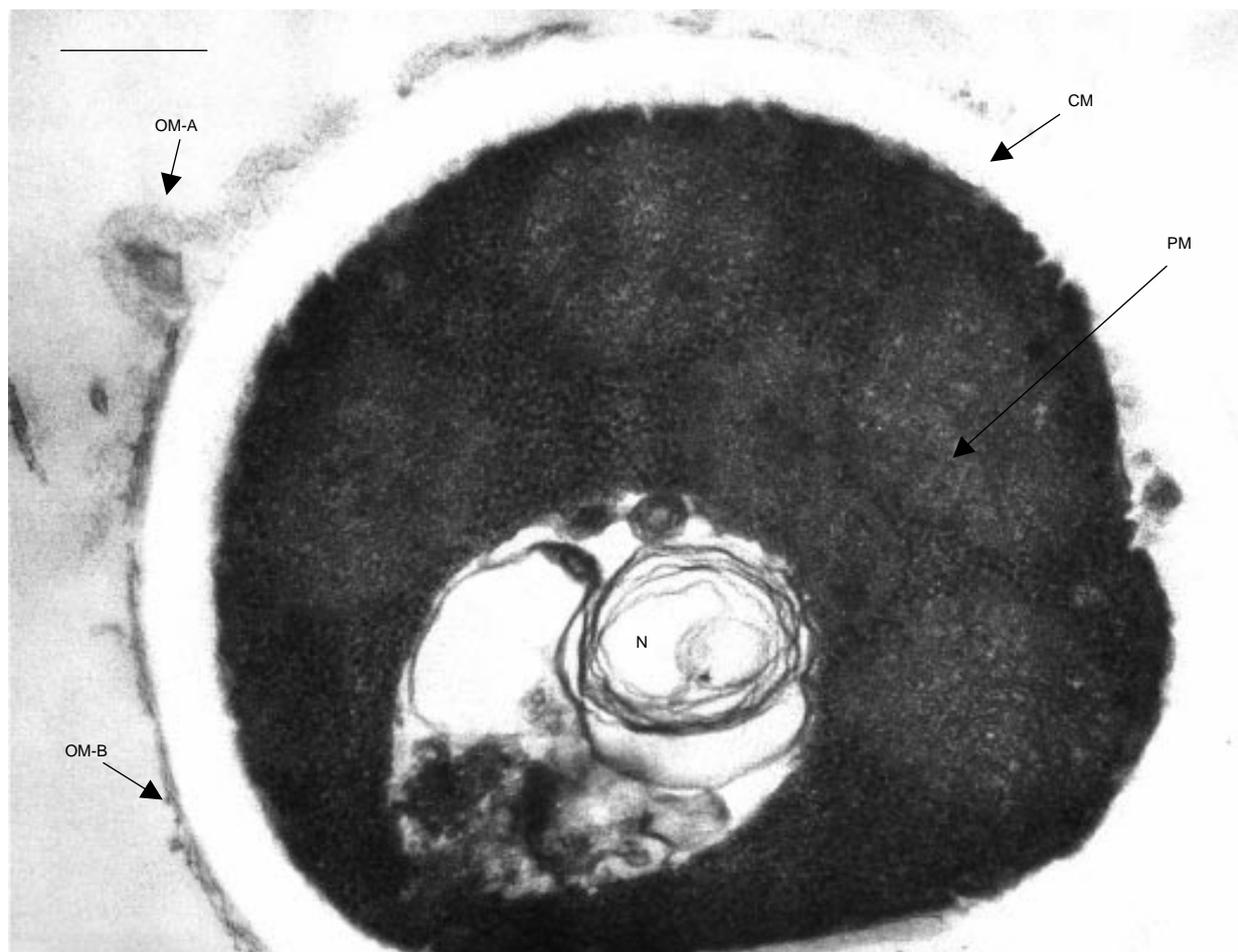
## RESULTS

**Concentration of *Aspergillus candidus* in the air of various facilities.** The concentration of *Aspergillus candidus* spores in the air of grain storing- and processing plants was large and significantly greater ( $p < 0.001$ ) compared to other facilities (Fig. 1). In the air of grain elevators and mills, the amount of *A. candidus* ranged from  $5.46 \times 10^4$  -  $6.47 \times 10^5$  cfu/m<sup>3</sup> and accounted for 93.2–99.0% of total fungi present in the air. All these concentrations exceeded

the Occupational Exposure Limit (OEL) value for airborne fungi proposed in Poland, equal to  $5.0 \times 10^4$  cfu/m<sup>3</sup> [12]. In the air of an animal feed processing facility, *A. candidus* occurred in the concentration of  $5.0 \times 10^3$  cfu/m<sup>3</sup> and formed 37.0% of total fungi. In the air of the facilities processing plant materials other than grain, the concentration of *A. candidus* was very small ( $1.0$ - $2.0 \times 10^2$  cfu/m<sup>3</sup>), except for sacking of canary grass where it



**Figure 3.** Abundant growth of *Aspergillus candidus* on a malt agar plate inoculated with a diluted ( $10^6$ ) extract of mouldy rye grain (sample marked as "D" in Figure 2), associated with ODS symptoms in exposed students.



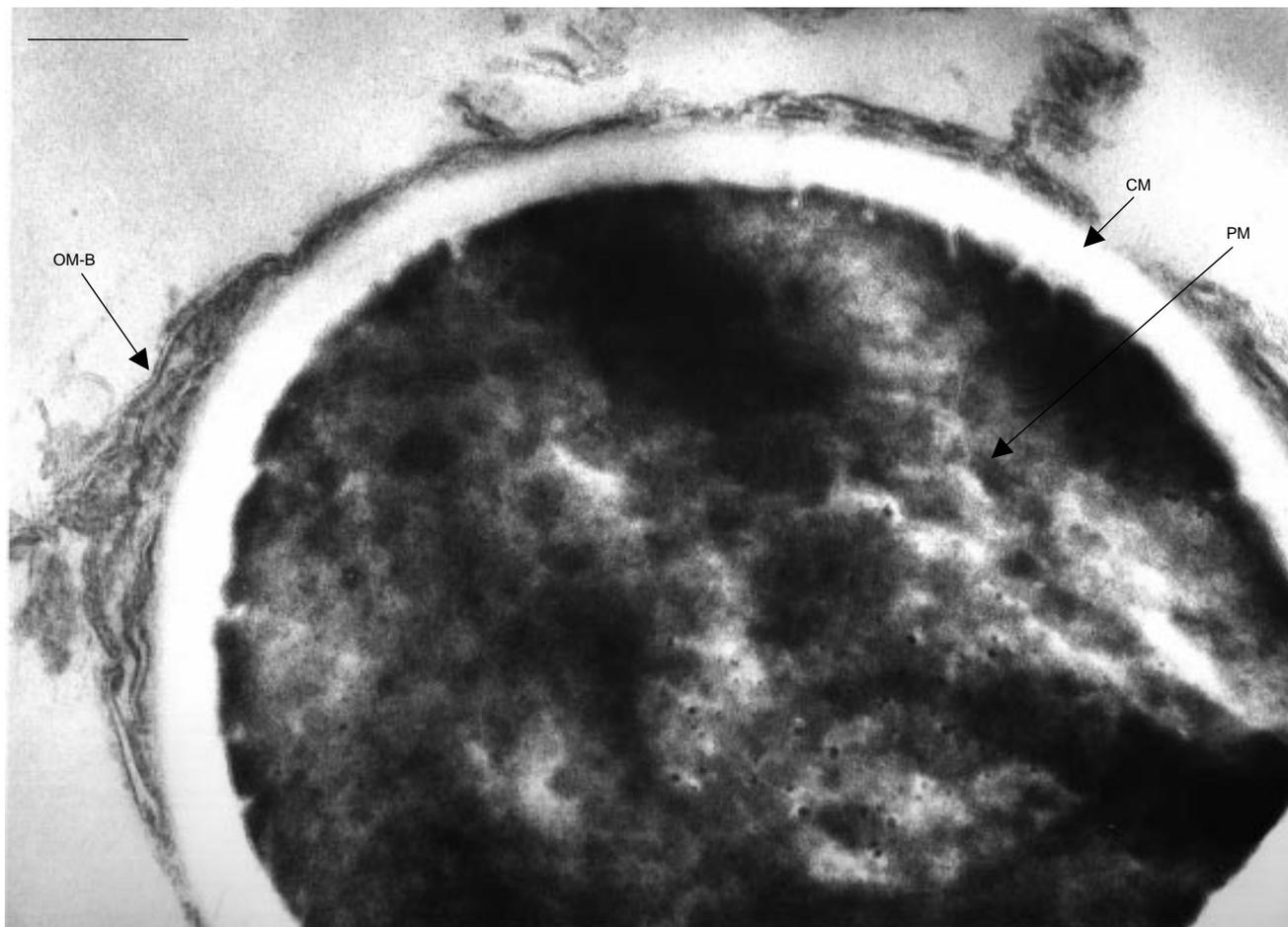
**Figure 4.** Ultrathin section of the spore of *Aspergillus candidus*, strain AC-III, EM. OM-A - external outer membrane, OM-B - internal outer membrane, CM - chitin membrane, PM - protoplasm, N - Nucleus. Bar represents 0.2  $\mu\text{m}$ . Photograph by Dr. Barbara Urbanowicz, Laboratory of Electron Microscopy, Institute of Pediatrics, Collegium Medicum of Jagiellonian University, Kraków.

was  $2.57 \times 10^4$  cfu/m<sup>3</sup>. Nevertheless, in contrast to grain processing plants, in all other facilities *Aspergillus candidus* constituted only a small fraction of the total fungal flora of the air (0.7–3.6%).

**Concentration of *Aspergillus candidus* in plant materials and settled dusts.** Similar to the air samples, the concentration of *A. candidus* in the samples of grain and grain dust was many times and significantly ( $p < 0.001$ ) greater than in the samples of other plant materials and dusts (Fig. 2). The concentration of this fungus in the barley and rye grain and in dust from barley and wheat was within a range of  $3.0 \times 10^5 - 3.0 \times 10^9$  cfu/g, forming 54.5–93.9% of total mycobiota. The highest concentration of *A. candidus* was found in a sample of barley dust associated with the ODTs cases among young people exposed to the dust (Fig. 3). Among other materials and dusts, the only result similar to those for grain and grain dust was noted in the case of straw sampled on the farm of a patient with allergic alveolitis. The concentration of *A. candidus* in this sample was  $2.5 \times$

$10^7$  cfu/g and constituted 94.5% of total fungi recovered from the sample. The concentration of this fungus in dust from oak wood processed in a sawmill was  $1.0 \times 10^5$  cfu/g and formed only 0.8% of total mycobiota. None *A. candidus* strains were found in the samples of canary grass and herbs.

**Ultrastructure of *Aspergillus candidus* spores.** It may be seen in Figure 4 that the spore, composed of protoplasm and nucleus, is surrounded with a distinct, electronically light chitin membrane (CM), about 120 nm (0.12  $\mu\text{m}$ ) wide. This membrane is unevenly covered by two fragmentary layers of outer membrane (OM-A, OM-B). The external, faint layer of outer membrane (OM-A) is composed of slightly visible ellipsoidal elements, measuring  $30 \times 50$  nm. The internal layer of outer membrane (OM-B) consists of fibrils possessing a distinct trilaminar structure (dense-light-dense), 5–15 nm wide. These fibrils, easily peeling off the spore surface, are very visible in the section of another spore (Fig. 5), where the external layer (OM-A) is virtually absent.



**Figure 5.** Ultrathin section of the spore of *Aspergillus candidus*, strain AC-III, EM. OM-B - internal outer membrane, CM - chitin membrane, PM - protoplasm. Bar represents 0.2  $\mu$ m. Photograph by Dr. Barbara Urbanowicz, Laboratory of Electron Microscopy, Institute of Pediatrics, Collegium Medicum of Jagiellonian University, Kraków.

**Effects of experimental exposure to inhalation of the extract of *Aspergillus candidus*.** Rabbits exposed to the long-term inhalation of the *Aspergillus candidus* extract showed, except for a slight rise after the first inhalation, a gradual decrease of mean migration index (MI) in the presence of the specific antigen (Fig. 6). The fall in the initial MI value ( $0.9955 \pm 0.1383$ ) appeared most distinct after 10th and 15th exposures, being respectively  $0.7348 \pm 0.0730$  and  $0.6329 \pm 0.0410$  ( $p < 0.01$ ). At the end of the experiment all the exposed animals showed a positive reactions to *A. candidus*, both in the MIF and precipitation tests. None of the control animals showed a positive reaction in either test or MI decrease.

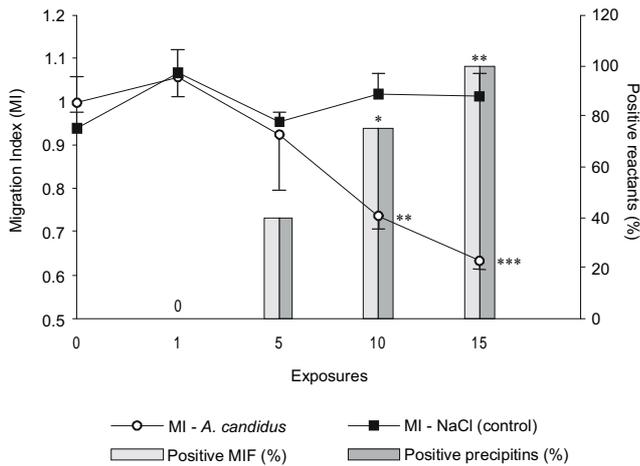
Inflammatory changes of diverse severity were found in the lungs of exposed rabbits. These differed from widening of interalveolar septa to haemorrhagic and exudative changes of soft lung tissue, and infiltrates of interalveolar septa and peribronchial tissue with mononuclear cells.

**Effects of experimental exposure to live spores of *Aspergillus candidus*.** The immunomodulating effects of

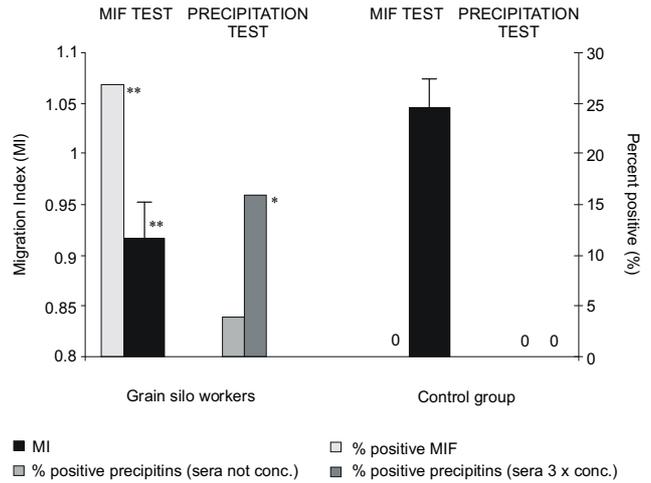
the repeated exposure to inhalation of live spores of *A. candidus* were stronger compared to those of the extract (Fig. 7). A rapid fall of the migration index was noted, from the initial value of  $1.1017 \pm 0.1503$  to  $0.8449 \pm 0.1245$  after the first exposure ( $p < 0.01$ ), and to  $0.5659 \pm 0.1159$  ( $p < 0.001$ ) after the second exposure. After the first exposure one-third of the rabbits showed a positive MIF reaction, and after the second exposure all the animals were highly positive. None of the rabbits exposed to *A. candidus* spores produced precipitins. All animals of the control group were negative, both in the MIF and precipitation tests.

**Immunological study of exposed human population.**

The average value of migration index in the group of grain workers exposed to the inhalation of *A. candidus* spores was significantly smaller compared to control subjects ( $p < 0.01$ ) (Fig. 8). Seven out of 26 grain workers (26.9%) showed a positive MIF reaction in the presence of specific *A. candidus* antigen, while no positive reaction was noted among controls ( $p < 0.01$ ). In the precipitation



**Figure 6.** Immunologic changes in rabbits exposed to long-term inhalation of the extract of *Aspergillus candidus*, assessed by the inhibition of leukocyte migration (MIF) and precipitation tests. Curves show changes of migration index (mean  $\pm$  S.E.) in the experimental and control groups of rabbits whilst bars indicate percentages of animals showing positive reactions in the MIF and precipitation tests. \* - \*\*\* - values significantly different from the initial value (0); \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .



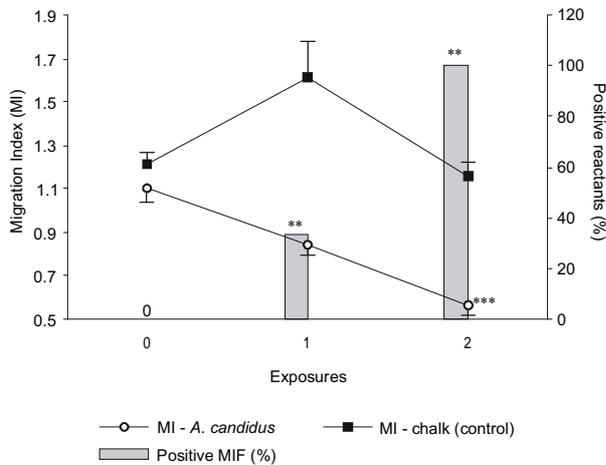
**Figure 8.** Cellular and humoral response of grain silo workers to the extract of *Aspergillus candidus*, compared to control group not exposed to organic dust. Cellular response is shown by the values of migration index (mean  $\pm$  S.E.) and by percent of positive reactants in MIF test. Humoral response is shown by percent of positive reactants in precipitation test. \* - \*\* - significant difference between the groups of grain silo workers and controls: \*  $p < 0.05$ , \*\*  $p < 0.01$ .

## DISCUSSION

The results of the present work show that the filamentous fungus *Aspergillus candidus* represents a potential respiratory hazard for grain workers. The spores of this white growing mould occur in large quantities in grain dust and in air contaminated with this dust. They possess strong immunostimulating properties, inducing a specific cellular and humoral response in experimentally exposed animals and in a part of grain workers occupationally exposed to this fungus.

Our results are in accordance with the recent results of Sorenson *et al.* [41], Shahan *et al.* [38, 39] and Nessa *et al.* [30], who demonstrated experimentally the non-specific, immunomodulating effects of this fungus shown by strong activation of complement and alveolar macrophages leading to release of cytokines and oxygen radicals. Stead *et al.* [44] found that *Aspergillus candidus* produces a prenylated p-terphenyl metabolite possessing potent cytotoxic activity; while Fischer *et al.* [14] reported a release of potentially harmful volatile compounds by this fungus. All these data indicate a strong inflammatory potential of *Aspergillus candidus*. To date, little research has been done on the specific response to *A. candidus* antigen. Tse *et al.* [46] reported that out of 40 Canadian elevator workers, 10 showed a positive reaction to *A. candidus* extract in intradermal test, and 1 revealed the presence of precipitins against this extract.

Weber *et al.* [49] suggest that the inflammatory reaction in the lungs after inhalation of organic dusts may be caused both by specific (allergic) and non-specific (immunotoxic) mechanisms. This sound statement most probably applies to the pathogenic action of *Aspergillus candidus*, where immunotoxic effects reported by the



**Figure 7.** Immunologic changes in rabbits exposed to the repeated inhalation of the spore powder of *Aspergillus candidus*, assessed by the inhibition of leukocyte migration (MIF) test. Curves show changes of migration index (mean  $\pm$  S.E.) in the experimental and control groups of rabbits whilst bars indicate percentages of animals showing positive reactions. \*\* - \*\*\* - values significantly different from the initial value (0): \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

test, 16% of positive reactions to *A. candidus* were found with 3-fold concentrated sera of grain workers and 4% with unconcentrated sera. In the control group, none precipitation reactions were found either with concentrated and unconcentrated sera. The difference between the two groups was significant in the case of concentrated sera ( $p < 0.05$ ).

above-mentioned authors [30, 38, 39, 41, 44] may be aggravated by specific activation of cellular response, found in the present work. The result could be a disorder of diverse pathogenesis, showing features both of ODTS and allergic alveolitis.

It is not entirely clear which components of *A. candidus* spore or mycelium may elicit a pathogenic reaction. The ultramicroscopic images of the spores suggest that these are easily detaching fragments of two outer membrane layers:

- the outermost layer composed of faint ellipsoidal globules (OM-A), corresponding to glucan supermacro-molecules;
- the layer composed of trilaminar fibrils (OM-B), corresponding to glycoproteins [1, 5].

Because of the small dimensions (usually below 0.2 µm), detaching fragments of both layers may easily penetrate down to the alveoli. In the light of contemporary knowledge, the specific reactions found in this work, and most of the non-specific ones, is probably caused by glycoprotein constituents of spore wall, while glucans may enhance the effects by non-specific immunotoxic activity [18, 35, 40, 42]. It must be stressed that our presumption concerning the nature of the immunologically active spore components has only a limited scientific value, as this has yet to be proved by immunolabelling, similar to the case of endotoxin-containing globules [13]. Nevertheless, the aim of presenting this hypothesis is to stimulate further research on this subject.

So far, the adverse effects of grain dust on human respiratory system have been attributed to various biological factors associated with grain, in particular to: thermophilic actinomycetes of the species *Saccharopolyspora rectivirgula* and *Thermoactinomyces vulgaris* developing in overheated grain [23, 48], Gram-negative bacteria *Pantoea agglomerans* and strong endotoxin produced by this species [12, 20, 22, 28, 29], coryneform bacteria *Arthrobacter globiformis* [29], some allergenic and mycotoxin-producing fungal species and genera: *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium citrinum*, *Fusarium graminearum*, *Stachybotrys atra* [9, 23, 28, 42, 48], fungi parasiting on wheat (*Puccinia graminis*, *Tilletia tritici*, *Ustilago avenae*) [23, 24], storage mites (*Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Glycyphagus domesticus*) and insects feeding on grain (*Sitophilus granarius*) [24, 28, 48]. The results of this work indicate that *Aspergillus candidus* should be added to this list and certainly placed among the top 10 of the harmful factors associated with the inhalation exposure to grain dust.

The prevention measures for limiting exposure of grain workers and farmers to *Aspergillus candidus* should include:

- proper storing of grain at low temperature and humidity,
- application of safe preservation chemicals,
- use of effective, positive pressure respirators which trap fine particles,

- health education, explaining the threat associated with handling mouldy grain.

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