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Commensal Fungi are Involved in Antigen-Specific Antibody Production in the Elderly

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Fungi are an important health hazard as commensal antigens. To demonstrate sensitization to fungi in the elderly and the influence of prohibition of oral intake under intravenous hyperalimentation (IVH) management with administration of antibiotics, we measured commensal fungus-specific antibodies.
Methodology: Thirty one college students (21.7±1.0 years): Young adult group, 28 elderly subjects over 75 years from the outpatient department (84.3±4.5 years): Outpatient group, and 21 elderly subjects over 75 years who were inpatients and required IVH (87.6±6.0 years): Inpatient group were enrolled. Plasma β-D-glucan and serum total immunoglobulin (Ig) E, antigen-specific IgE for house dust (HD), *Cladosporium*, *Alternaria*, *Trichophyton*, and *Candida* and *Candida*-specific IgG were measured.
Results: Total IgE level was significantly decreased in the outpatient group compared to the young

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adult group ($p<0.01$), and was increased in the inpatient group compared to the outpatient group ($p<0.05$). HD-specific IgE was elevated in the young group compared to the two elderly groups ($p<0.01$, respectively). There was no tendency for detection of *Cladosporium*-or *Alternaria*-specific IgE in the three groups. *Tricophyton*-specific IgE level was significantly elevated in the inpatient group compared to the young adult group ($p<0.01$). *Candida*-specific IgE level was significantly elevated in the inpatient group compared to the outpatient group ($p<0.05$). *Candida*-specific IgG was significantly elevated in the inpatient group compared to the other two groups ($p<0.001$, respectively).

Conclusion: It is suggested that commensal fungi, such as *Trichophyton* and *Candida*, are more markedly associated with antigen-specific immunoglobulin production in an immunocompromised condition in the elderly.

Keywords: Candida-specific IgE; Candida-specific IgG; commensal fungi; elderly; microflora hypothesis; Trichophyton-specific IgE.

1. INTRODUCTION

Fungal spores and mycelial cells are ubiquitous in the environment and are also known to be common human saprophytes. Fungi infect organs, produce toxins, and generate harmful immune responses, such as allergic diseases [1].

Immunosenescence is thought to play a role in the prevalence and severity of allergic sensitization in the elderly [2]. It has been consistently observed that allergen sensitization evaluated by total IgE and allergen-specific IgE declines with age [3]. Another study demonstrated that the allergy epidemic has spread to older adults [4].

The authors previously published data on the prevalence of *Trichophyton* and *Candida* detection in elderly persons [5]. This study aimed to demonstrate the relationship between aging and sensitization to fungi. We measured antigen-specific antibodies to fungi in the same subjects. The influence of prohibition of oral intake under intravenous hyperalimentation management with antibiotic use in the elderly on the prevalence of commensal fungus-specific antibodies is also discussed.

2. MATERIALS AND METHODS

2.1 Subjects

The following subjects were enrolled in the study: Young adult group: 31 college students, 21-25 years, mean 21.7±1.0 years; male 6, female 25, outpatient group: 28 elderly subjects over 75 years from the outpatient department, 75-95 years, mean 84.3±4.5 years; male 9, female 19, and inpatient group: 21 elderly subjects over 75 years who were inpatients, 75-101 years, mean 87.6±6.0 years, male 4, female 17. All subjects in the inpatient group showed disuse atrophy and were bedridden. They were not allowed oral intake and were under IVH management. The duration of an indwelling central venous catheter was 81.5±33.4 (50 to 183) days, that of intravenous hyperalimentation was 78.5±33.7 (44 to 182) days, and that of prohibition of food and drink was 78.3±23.2 (52 to 144) days. All of the patients had been administered antibiotics during their hospital stay. Patients who had received continuous or intermittent corticosteroids, immunosuppressants, or antineoplastic drugs were excluded from the study. Subjects with clinical signs and symptoms of proven deep-seated candidosis or systemic candidosis, and subjects with serum concentration of β-D-glucan over 20 pg/ml were excluded. All samples were collected after obtaining informed consent. This study was approved by the Ethics Committee of Akishima Hospital.

2.2 Measurement of Plasma β-D-glucan

β-D-glucan level of each subject was measured with Fungitec G Test MK® according to the manufacturer's recommendations (Seikagaku Biobusiness Corporation, Tokyo, Japan). The cut-off value of β-D-glucan level for diagnosis of mycosis is 20 pg/mL. A level of 10-20 pg/mL requires observation, and a level below 10 pg/mL is normal. Subjects with a value below 20 pg/mL were included in this study.

2.3 Measurement of Serum Immunoglobulin Concentrations

Blood serum was collected for testing. Immunoglobulin was determined in the same frozen samples.

2.4 Total IgE

Total IgE was measured by fluoroenzymeimmunoassay using ImmunoCAP® Total IgE (Phadia, Uppsala, Sweden) according to the recommendations of the manufacturer.

2.5 Antigen-specific IgE

Serum levels of HD, *Alternaria*, *Cladosporium*, *Tricophyton*, and *Candida*-specific IgE antibodies were analyzed with UniCAP®, according to the recommendations of the manufacturer (Phadia, Uppsala, Sweden). A value ≥0.35 unit of allergen (UA)/ml was considered positive for specific IgE.

2.6 Antigen-specific IgG

Quantification of *Candida*-specific IgG was performed with the UniCAP® 100 system (Sweden Diagnostics), according to the manufacturer's instructions. Results were expressed as milligrams of antigen-specific antibodies (mgA/L).

2.7 Statistical Analysis

Values are expressed as mean ± SD, genomic mean or median, and range. The geometric mean, rather than the arithmetic mean, was used to approximate the normal distribution for statistical inference and modeling. For analysis, log 10 transformation was used to obtain normally distributed data for total IgE and *Candida*-specific IgG. Welch's *t*-test (two-tailed) was employed for analysis of total IgE and *Candida*-specific-IgG. Mann-Whitney U test was used for analysis of β-D-glucan and allergen-specific IgE. Values of $p<0.05$ were considered to indicate statistically significant differences.

3. RESULTS

3.1 Distribution of β-D-glucan

Although all the data were below the clinical cut-off level for deep-seated mycosis, β-D-glucan level was significantly higher in the inpatient group (median: 6.8, range: <5 to 19 pg/ml) compared to both the young adult group (median: <5, range: <5 to 14 pg/ml) ($p<0.05$) and outpatient group (median: <5, range: <5 to 17 pg/ml) ($p<0.05$) Fig. 1.

3.2 IgE RIST

Total IgE level was significantly decreased in the outpatient group (geometric mean: 33.05, range: 2.34 to 438 IU/ml) compared to the young adult group (geometric mean: 95.39, range: 3.98 to 1020 IU/ml) ($p<0.01$). It was significantly increased in the inpatient group (geometric mean: 100.56, range: 5.89 to 1836 IU/ml) compared to the outpatient group ($p<0.05$) Fig. 2.

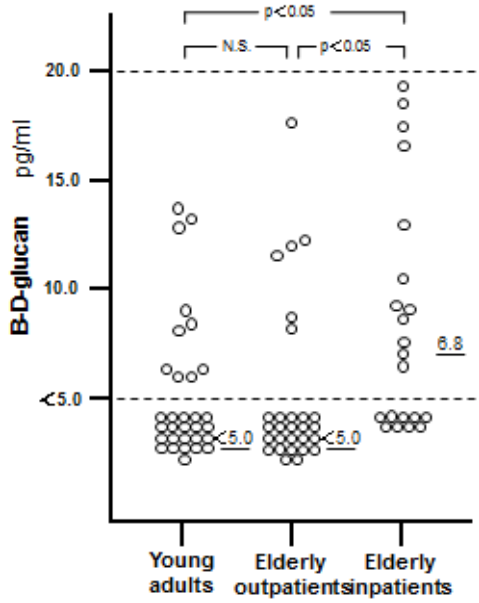

Fig. 1. β-D-glucan in young adults, elderly outpatients and elderly inpatients

Clinical cut-off level for deep-seated mycosis is 20 UA/ml and is indicated as broken line. Bars represent median. β-D-glucan level was significantly higher in the inpatient group compared to both the young adult group and outpatient group

3.3 Allergen-specific IgE

Subjects with elevated HD-specific IgE were significantly more frequent in the young adult group (median: 0.49, range: <0.35 to 54.6 UA/ml) compared to the outpatient group (median: <0.35, range: <0.35 to 2.32 UA/ml) and inpatient group (median: <0.35, range: <0.35 to 6.37 UA/ml) ($p<0.01$, respectively). *Cladosporium*-specific IgE level was not significantly different among the three groups: young adult group (median: <0.35, range: <0.35 to <0.35 UA/ml), outpatient group (median: <0.35, range: <0.35 to <0.35 UA/ml), and inpatient group (median: <0.35, range: <0.35 to 1.24 UA/ml). Similarly, *Alternaria*-specific IgE level was not significantly different in each group: young adult group (median: <0.35, range: <0.35 to 4.80 UA/ml), outpatient group (median <0.35, range: <0.35 to <0.35 UA/ml), and inpatient group (median: <0.35, range: <0.35 to 0.53

UA/ml). *Tricophyton*-specific IgE was significantly increased in the inpatient group (median: <0.35, range: <0.35 to 28.0 UA/ml) compared to the young adult group (median: <0.35, range: <0.35 to 0.82 UA/ml) ($p<0.01$). The same tendency was observed compared to the outpatient group (median: <0.35, range: <0.35 to 2.83 UA/ml), but it was not statistically significant. Although a statistically significant difference was not shown, there was a tendency for *Trichophyton*-specific IgE to be elevated in the outpatient group compared to the young adult group. Significantly, a higher *Candida*-specific IgE level was observed in the inpatient group (median: <0.35, range: <0.35 to 4.69 UA/ml) compared to the outpatient group (median: <0.35, range: <0.35 to 0.38 UA/ml) ($p<0.05$). There was a tendency for *Candida*-specific IgE to be elevated in the inpatient group compared to the young adult group (median: <0.35, range: <0.35 to 0.44 UA/ml), but this was not statistically significant Fig. 3.

3.4 Comparison of *Candida*-specific IgG Distribution

In the inpatient group (geometric mean: 212.94, range: 28.7 to 1268 mgA/L), *Candida*-specific IgG level was significantly higher than that in both the young adult group (geometric mean: 27.70, range: 2.29 to 482 mgA/L) and outpatient group (geometric mean: 40.09, range: 3.75 to 178 mgA/L) ($p<0.001$, respectively) Fig. 4.

3.5 Clinical Allergy Symptoms

Clinical manifestations of *Tricophyton*- or *Candida*- related allergy were not currently present in any subject.

4. DISCUSSION

Spores, hyphae and fungal fragments contribute to exposure and allergic sensitization. Protease activity of fungi is also now thought to play roles in immune responses by inducing disruption of the tight junctions between epithelial cells, activation of protease-activated receptor-2 (PAR-2), and the production of thymic stromal lymphopoietin (TSLP). These facilitate allergen delivery across epithelial layers and enhance allergenicity or directly activate the immune system through a non-allergic mechanism [6]. Data on exposure and sensitization to fungal allergens are still limited to the assessment of a few select and easily identifiable species. The optimal growth conditions vary among different molds, and this complicates the analysis and evaluation of the rate of detection in the environment and involvement in human disease. A major difference to other allergens, such as house dust, mites and pollen, is that fungi may colonize the human body, often translocating and spreading throughout the body.

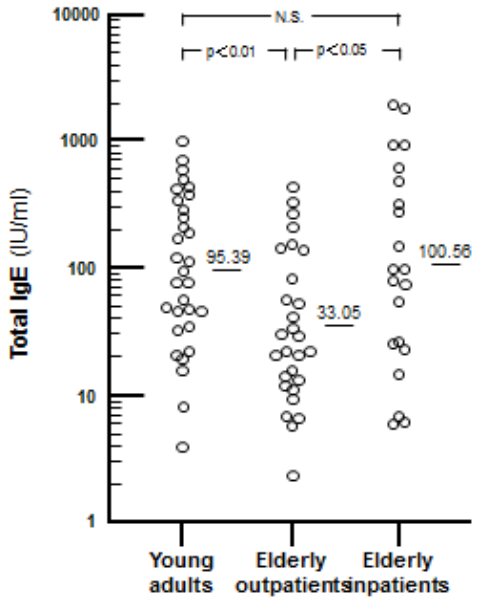

Fig. 2. Total IgE titre in young adults, elderly outpatients and elderly inpatients

Bars represent geometric mean. Total IgE level was significantly decreased in the outpatient group compared to the young adult group, reflecting an age-dependent decline. However, in the inpatient group, total IgE level was significantly increased compared to that in the outpatient group, suggesting immune reactions to certain antigens

Alternaria and *Cladosporium* are major abundant fungi in both the outdoor [7] and indoor environment [8]. Like pollens, they are often found at high levels indoors if there is access to outdoor air. *Alternaria* is an important factor in asthma, including thunderstorm-related asthma [9-11]. The involvement of *Cladosporium* in asthma also has been recognized clinically and epidemiologically [12]. Thus, *Alternaria* and *Cladosporium* are known to be associated with significant human allergy [13]. However, in our study, the titers of *Alternaria* and *Cladosporium*-specific IgE were not elevated in the two elderly groups compared to the young adult group.

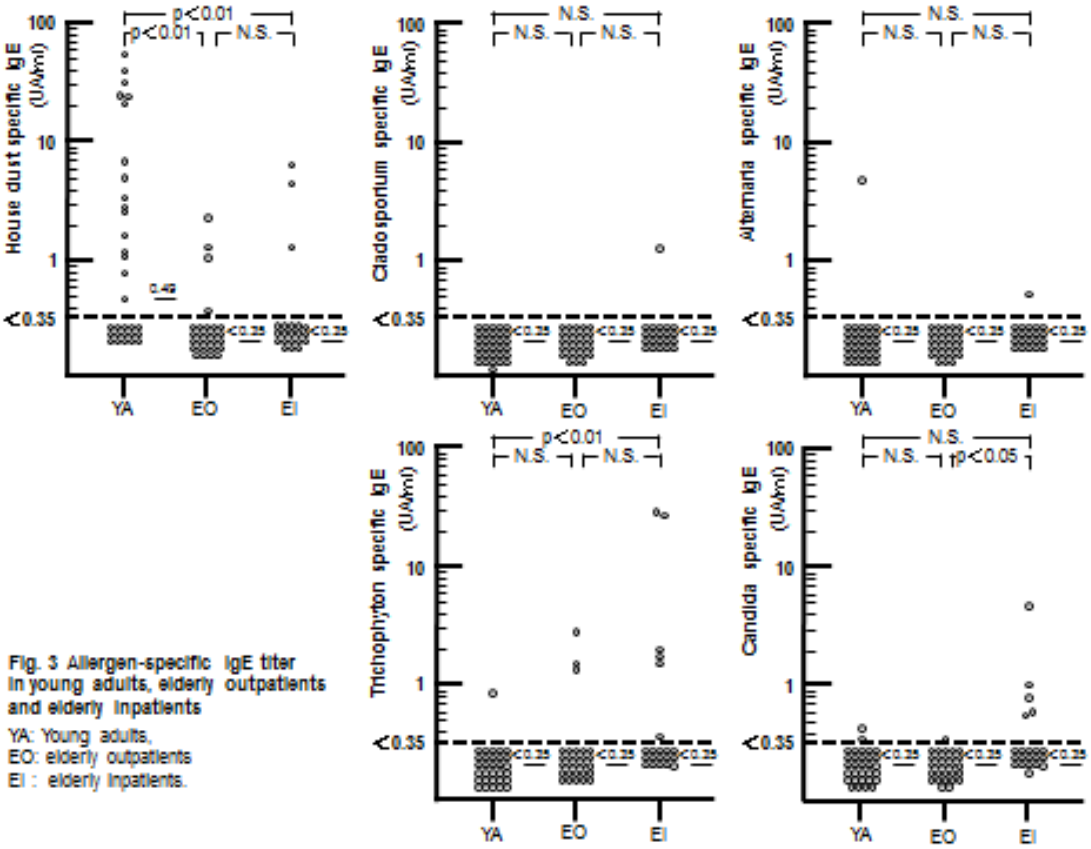

Fig. 3. Allergen-specific IgE titre in young adults, elderly outpatients and elderly inpatients

The cut-off level of 0.35 UA/ml is indicated as a broken line. Bars represent median. HD-specific IgE was elevated in the young adult group compared to that in the two elderly groups. Tricophyton-specific IgE was significantly increased in the inpatient group compared to that in the young adult group. Candida-specific-IgE level was significantly elevated in the inpatient group compared to that in the outpatient group. Exposure to residential fungi plays a significant role in sensitization and antigen-specific IgE production

The prevalence of opportunistic fungal infections has increased markedly in the aged population in recent years. Aging, neglected hygiene, and immobilisation may contribute to the increased prevalence of fungal detection in elderly persons. Dermatophytosis occurring in later life manifests most frequently as *Trichophyton rubrum* infection of the toenails and plantar surfaces of the feet. Our previous toenail data using a PCR method in these same subjects demonstrated a significant difference in prevalence of nail *Trichophyton* between the young adult group (0.0%) and the outpatient group (35.7%, $p<0.01$) and inpatient group (57.1%, $p<0.01$) [5]. Decreased personal care, epidermal turnover and immune function with aging are risk factors for chronic dermatophytosis. There is evidence supporting a link between dermatophytosis and allergic diseases [14], urticaria, and atopic dermatitis. Although antibodies are detected in infected individuals [15], humoral immunity to dermatophytons is reported to be not protective. On the other hand, *Trichophyton*-specific IgE is observed in patients with Trichophytosis regardless of atopy [16]. In our study, *Trichophyton*-specific IgE tended to be elevated in the elderly group, and it was significantly higher in the inpatient group compared to the young adult group, suggesting the importance of maintaining hygiene and that specific IgE production occurs with long-term exposure and infection.

Candida albicans (*C. albicans*) is a common and harmless commensal of the human skin, nasopharynx, oral and gastrointestinal mucosa, and vaginal mucosa, and causes not only opportunistic infections in immunocompromised patients but also allergic reactions in people sensitized to *C. albicans*. Several studies suggest that about a quarter, or even more, of women with recurrent vulvovaginal candidosis

could have an allergic component contributing to the development and/or severity of their disease [17]. We have reported that the prevalence of *Candida* spp. was significantly higher in the outpatient group (18/28: 64.3%) and inpatient group (12/21; 57.1%) than in the young adult group (5/31; 16.1%) ($p<0.01$, respectively), by analysis of tongue swabs [5]. Several studies have supported *Candida* infections in candidemia to be of gastrointestinal origin, based on experimental, clinical, and molecular similarity studies [18,19]. Prohibited oral intake could disrupt the mechanisms involved in the development of immunological tolerance. Widespread use of antibiotics, which alter the physiological, competitive bacterial gut flora [20], and invasive medical instrumentation, such as with devices, long-term urinary catheters, and central venous catheters, have been implicated in the increased occurrence of fungal disease in the hospital environment [18]. Thus, critically ill elderly people are at increased risk of fungal translocation. Our data indicated that β-D-glucan and *Candida*-specific IgE were elevated in the inpatient group. Changes in the fungal and bacterial microbiota may be a factor involved in sensitization [21].

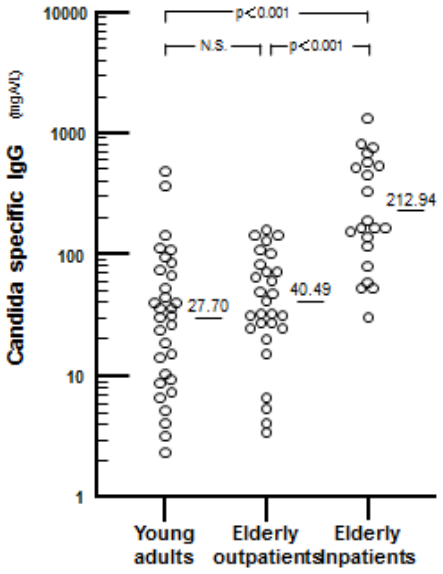

Fig. 4. Candida-specific IgG in young adults, elderly outpatients and elderly in patients

Bars represent geometric mean. In the inpatient group, Candida-specific IgG level was significantly elevated compared to that in both the young adult group and the outpatient group. The role of antigen-specific IgG was not clear

IgG is involved in anaphylaxis [22]. An experimental model of allergic conjunctivitis demonstrated that continuous topical antigen challenge induced IgG1/IgG2 production following activation and down-regulation of mast cells, IgE production, mast cell degranulation and exhaustion, histamine release, lymphoid hyperplasia and angiogenesis [23]. Allergen-specific IgG may promote expansion of the secondary Th2 response through ligation of FcγRs on innate immune cells [24], and be involved in the development of airway hyperresponsiveness [25], suggesting that allergen-specific IgG could play an important role in allergic diseases. However, it remains controversial whether IgG contributes to the pathogenesis of or tolerance to allergy. In school children, allergy was associated with IgG antibodies to molds that can be found in moisture-damaged buildings. However, no association was found between IgG antibodies to molds and exposure to moisture and molds [26].

Candida-specific Igs have been reported in patients with systemic candidosis [27]. *Candida*-specific IgG concentration was significantly higher in patients with increased Fungus Related Disease Questionnaire (FRDQ-7) scores, suggesting that people with Candida syndrome have a score >9 [28]. All elderly inpatients in this study were administered broad spectrum antibiotics during their hospitalization, so their FRDQ-7 scores were estimated to be ≥6, falling in the “probable FRD”. In the inpatient group, *Candida*-specific IgG level was significantly elevated compared to that in both the young adult group and the outpatient group. There was no association between *Candida*-specific IgE and IgG. The role of *Candida*-specific IgG antibodies, which could represent allergic sensitization or, on the other hand, only mold exposure or protection against infection, is not clear.

With the increase of elderly persons, evaluation of antigen sensitization is an important issue. Cross-sectional studies have shown that the prevalence of atopy decreases with increasing age. An age-related decline in serum total IgE has been reported [3]. Defects in T-cell activation may result in decreased availability of IL-4 and the waning of IgE responses [29]. Decreased IgE levels may also occur through altered activities of mast cells due to changes in the immune response with the aging process [30,31]. The prevalence of elevated antigen-specific IgE with aging has also been reported [32], with some studies suggesting heterogeneity for each

allergen [33]. Atopy in middle-aged men increased during the last quarter of the 20th century [34], and the prevalence of atopy does not decline with increasing age [4].

Although epidemiological data have suggested that allergic diseases are associated with childhood antibiotic use and an altered intestinal microbiota, no study has examined allergic sensitization in elderly persons administered antibiotics. Early life exposure to environmental microorganisms is protective [35], and early life as well as in utero antibiotic exposure increases the risk of allergic asthma [36-37]. It is hypothesized that fungal exposure resulting in colonization or infection influences the tendency of an individual to develop allergic disease [38], suggesting that once the immune system is disrupted and dysregulated, allergic diseases can result. In mice, antibiotic therapy and an increased fungal microbiota resulted in the development of pulmonary allergic responses [20].

Production of antibodies that are specific for allergens is an important pathological process in inflammatory allergic diseases. Although immune function declines with aging, there is a possibility that exposure to residential fungi rather than environmental exposure to airborne molds is the main determinant of fungus-specific immune responses in immunosenescent elderly.

5. CONCLUSION

Molds are widely distributed in our living environment and are resident in the living body, and may detrimentally affect health in the elderly and individuals with a change in body condition, especially an immunocompromised state. Although allergies are often thought to be a reaction in childhood and young age, the initial production of antigen-specific immunoglobulin can occasionally appear in the elderly. Exposure to residential fungi plays a significant role in skewing the immune response toward sensitization, often without any clinical allergic manifestations. Host factors, rather than environmental exposure, are the main determinant of production of fungus-specific antibodies in the elderly. Long-term exposure and infection may be important in skin route sensitization, and disruption of the microbiota plays a role in sensitization via the digestive tract in elderly people.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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