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

Yasuhiro Matsumura1*, Michiko Abe2 and Koichi Makimura3

1Department of Internal Medicine, Akishima Hospital, Tokyo, Japan.
2Department of Medical Laboratory Sciences, School of Allied Health Sciences, Kitasato University, Kanagawa, Japan.
3Laboratory of Space and Environmental Medicine, Graduate School of Medicine, Teikyo University, Tokyo, Japan.

Authors’ contributions

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

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
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 Tricophyton-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.
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 dermatophytosis 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].

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 IgG 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.

ACKNOWLEDGEMENTS

We thank Phadia K.K. for their technical assistance with IgE and IgG measurement.

GRANTS

This study was supported in part by a Health Science Research Grant for Research on Emerging and Re-emerging Infectious Diseases, H25-Shinko-Ippan-006, from the Ministry of Health, Labour and Welfare of Japan (to K. M.).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Simon-Nobbe B, Denk U, Pölö V, Rid R, Breitenbach M. The spectrum of fungal allergy. Int Arch Allergy Immunol. 2008; 145(1):58-86.
2. Mathur SK. Allergy and asthma in the elderly. Semin Respir Crit Care Med. 2010;31(5):587-95.
3. Viswanathan RK, Mathur SK. Role of allergen sensitization in older adults. Curr Allergy Asthma Rep. 2011;11(5):427-33.
4. Zaui D, Bortolotti R, Grassi A, Tiberio D, Bianchi FB. Changes in atopy over 25 years: atopy now affects wider age range. BMJ. 2005;331(7512):352.
5. Matsumura Y, Abe M, Makimura K. Prevalence of Trichophyton and Candida in elderly persons. Health MED. 2013;7(5):1511-18.
6. Matsumura Y. Role of allergen source-derived proteases in sensitization via airway epithelial cells. J Allergy (Cairo). 2012;2012:903659.
7. Inal A, Karakoc GB, Altintas DU, Pinar M, Ceter T, Yilmaz M, et al. Effect of outdoor fungus concentrations on symptom severity of children with asthma and/or rhinitis monosensitized to molds. Asian Pac J Allergy Immunol. 2008;26(1):11-7.
8. Chew GL, Rogers C, Burge HA, Muilenberg ML, Gold DR. Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics. Allergy. 2003;58(1):13-20.
9. Bush RK, Prochnau JJ. Alternaria-induced asthma. J Allergy Clin Immunol. 2004;113(2):227-34.
10. Salo PM, Arbes SJ Jr, Sever M, Jaramillo R, Cohn RD, London SJ, et al. Exposure to Alternaria alternata in US homes is associated with asthma symptoms. J Allergy Clin Immunol. 2006;118(4):892-8.

11. Nasser SM, Pulimood TB. Allergens and thunderstorm asthma. Curr Allergy Asthma Rep. 2009;9(5):384-90.

12. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: A summary of the evidence. Eur Respir J. 2006;27(3):615-26.

13. Tariq SM, Matthews SM, Stevens M, Hakim EA. Sensitization to Alternaria and Cladosporium by the age of 4 years. Clin Exp Allergy. 1996;26(7):794-8.

14. Escalante MT, Sánchez-Borges M, Capriles-Hulett A, Belfort E, Di Biagio E, González-Aveledo L. Trichophyton-specific IgE in patients with dermatophytosis is not associated with aeroallergen sensitivity. J Allergy Clin Immunol. 2000;105(3):547-51.

15. Williams JW, Tjota MY, Sperling AI. The contribution of allergen-specific IgG to the development of Th2-mediated airway inflammation. J Allergy (Cairo). 2012;2012:236075.

16. Oshiba A, Hamelmann E, Takeda K, Bradley KL, Loader JE, Larsen GL, et al. Passive transfer of immediate hypersensitivity and airway hyperresponsiveness by allergen-specific immunoglobulin (Ig) E and IgG1 in mice. J Clin Invest. 1996;97(6):1398-1408.

17. Taskinen TM, Laitinen S, Nevalainen A, Vepsäläinen A, Meklin T, Reiman M, et al. Immunoglobulin G antibodies to moulds in school-children from moisture problem schools. Allergy. 2002;57(1):9-16.

18. Bär W, Hecker H. Diagnosis of systemic Candida infections in patients of the intensive care unit. Significance of serum antigens and antibodies. Mycoses. 2002;45(1-2):22-8.

19. Lewith GT, Chopra S, Radcliffe MJ, Abraham N, Prescott P, Howarth PH. Elevation of Candida IgG antibodies in patients with medically unexplained symptoms. J Altern Complement Med. 2007;13(10):1129-33.

20. al-Rayes H, Pachas W, Mirza N, Ahern DJ, Geha RS, Vercelli D. IgE regulation and lymphokine patterns in aging humans. J Allergy Clin Immunol. 1992;90(4Pt 1):630-6.

21. Khatami M. Inflammation, aging, and cancer: Tumoricidal versus tumorigenesis of immunity: a common denominator mapping chronic diseases. Cell Biochem Biophys. 2009;55(2):55-79.

22. Wüthrich B, Schindler C, Medici TC, Zeilweger JP, Leuenberger P. IgE levels, atopy markers and hay fever in relation to age, sex and smoking status in a normal adult Swiss population. SAPALDIA (Swiss Study on Air Pollution and Lung Diseases in Adults) Team. Int Arch Allergy Immunol. 1996;111(4):396-402.
total IgE with age and cohort. J Allergy Clin Immunol. 2005;116(3):675-82.
34. Law M, Morris JK, Wald N, Luczynska C, Burney P. Changes in atopy over a quarter of a century, based on cross sectional data at three time periods. BMJ. 2005;330(7501):1187-8.
35. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrländer C, et al. GABRIELA Transregio 22 Study Group. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011;364(8):701-9.
36. Marra F, Marra CA, Richardson K, Lynd LD, Kozyrskyj A, Patrick DM, et al. Antibiotic use in children is associated with increased risk of asthma. Pediatrics. 2009;123(3):1003-10.
37. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. EMBO Rep. 2012;13(5):440-7.
38. Goldman DL, Huffnagle GB. Potential contribution of fungal infection and colonization to the development of allergy. Med Mycol. 2009;47(5):445-56.

© 2015 Matsumura et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?id=719&id=12&aid=7056