Onychomycosis from Aspergillus melleus, a Novel Pathogen for Humans. Fungal Identification and in vitro Drug Susceptibility

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Background

Aspergillus P. Micheli ex Haller is a genus of filamentous microfungi usually saprotrophs, ubiquitous, occurring in the environment. There are 339 species of Aspergillus (1).

In medical mycology, Aspergillus spp. have been described as agents of both systemic and superficial infections. Aspergillus may cause a broad spectrum of systemic diseases in humans, ranging from hypersensitivity reactions to direct angioinvasion. Superficial and local infections include otomycosis, tracheobronchitis, primary and secondary cutaneous infections and onychomycosis (s1). In fact, Aspergillus spp are the second most commonly recovered species among non-dermatophytic moulds in Europe (s2).

Questions addressed

Aspergillus spp. occurring in onychomycosis are often the same as involved in systemic infections, such as Aspergillus flavus, A. nidulans, A. terreus and A. versicolor (s3–s9). Recently, a number of Aspergillus onychomycosis have been described and several Aspergillus species (A. sclerotorium, A. tamari, A. sydowii, A. persii; A. nomius) have now been identified as onychomycosis agents (s6, 2–5). As opportunistic Aspergillus spp. have recently been reported as being capable of expanding their habitat by attacking human tissues, also in immunocompetent patients, it is necessary to recognize the species in order to administrate specific therapies.

Experimental design

We diagnosed onychomycosis, caused by A. melleus, in a 68-year-old Caucasian man through morphological and molecular identification. He presented with an extensive involvement of the nail plate with pseudo-leukonychia dots localized in the proximal portion of the fourth toenail on the right foot, developed several years before (Fig. 1). The patient underwent several unsuccessful treatments (topical treatments and itraconazole pulse therapy) during 3 years.

We performed a direct microscopic examination and macro- and micromorphological fungal identification with modified Sabouraud medium and Czapek yeast agar. Then, the fungal sequence was obtained by DNA amplification and deposited and compared to those available in the GenBank database (http://www.ncbi.nlm.nih.gov/). Drug susceptibility tests were performed (microdilution and Sensititre YeastOne® methods). Fungal identification and drug susceptibility methods are described in Data S1.

Results

Morphological characters

Direct microscopic examination revealed mycelial filaments (Fig. S1). Colony diameters at 7 days were (mm) as follows: CYA25 25–50; MEA 30–56; CYA37 20–40; CYA20S 57–65; and CZ 15–35. In CYA 25°C, the colony was golden yellow, plane or radially sulcate, and velutinous, with white vegetative mycelium and abundant conidial structures; colony reverse varied from pale orange to brown; soluble pigment presented as pale brown; and numerous sclerotia presented as yellow to pale brown. Microscopic character sizes are reported using three numbers corresponding to the minimum/average/maximum values. Conidial heads were biseriate and radiate; stipes appeared as light brown, roughened and 200/380/750 μm in length; spherical vesicles were 18/22/40 μm. Conidia were globose to subglobose, smooth to finely roughened and 2.8/3.3/4.2 μm. The strain was identified as Aspergillus melleus, a species belonging to the subgenus Circumdati (1.6) section Circumdati, the A. ochraceus group (7). The Circumdati section traditionally encompasses species with predominantly biseriate stigmata and small, yellow buff to ochre conidia; sclerotia that do not turn black (6); and ubiquinone systems Q-9, Q-10, and Q-10 (H2) (s12–s14).

Molecular results

Our sequence clusters (Fig. 2) with the A. melleus sequences present in the data set.

In vitro antifungal susceptibility

Microdilution method revealed that voriconazole (MIC = 0.12 μg/ml), posaconazole (MIC = 0.125 μg/ml), itraconazole (MIC = 0.25 μg/ml), amphotericin B (MIC = 2 μg/ml) and terbinafine (MIC = 0.5 μg/ml) showed a clear antifungal activity.

The YeastOne® panel showed that A. melleus was susceptible to voriconazole (MIC = 0.5 μg/ml), posaconazole (MIC = 0.5 μg/ml), itraconazole (MIC = 0.25 μg/ml) and amphotericin B (MIC = 1 μg/ml) and resistant to fluconazole, anidulafungin, caspofungin, micafungin and fluconazole.
Conclusion

Aspergillus spp. usually affect immunocompromised hosts, but are becoming increasingly prevalent in healthy people. This apparent emergence might be an artefact of improved diagnostic techniques or of an increased awareness that these fungi are potential aetiological agents (s20). As they are not usually keratinolytic, they are usually found as secondary invaders of the nail plate (s21, s22).

Aspergillus melleus has never been described as a pathogen for humans before. According to the phylogenetic tree (Fig. 2), our sequence from MUT 4199 falls in a clade (the A. melleus clade) together with four A. melleus sequences retrieved from GenBank (type strain NRRL 5103 included). The pairwise identity value of the β-tubulin sequences of the entire A. melleus clade is 99.2%.

Aspergillus melleus is quite common in soils and in the rhizosphere of tropical, subtropical or warm temperate regions. It has also been isolated from peanuts and different types of corn (s23). A. melleus and other Circumdati are known for their production of mycotoxins (8,9). They can also cause infections, although infrequently. A. ochraceus, A. sclerotiorum and A. persii have been described as agents of onychomycosis (4,8,9). A. westerdijkiae, A. insulicola A. tritici and A. pallidofulvus have also been recognized as pathogens for humans (6). Other species of the same section, such as A. tanneri, have been described as causing invasive infections (s15). Unfortunately, a standard treatment for onychomycosis due to Aspergillus spp. has not been established and a perfect cure is rather difficult to obtain. Aspergillus spp. are reported to have excellent susceptibility to itraconazole followed by miconazole, ketoconazole, tioconazole, fenticonazole and terbinafine (s25). Some authors have described Aspergillus as being resistant to terbinafine (s21, s22, s24). In our case, there was an agreement between the two methods used and a good susceptibility to azoles, amphotericine and terbinafine was shown (except for fluconazole). Moreover, the microdilution revealed sensitivity to terbinafine.

In conclusion, we describe the first case of Aspergillus melleus as a pathogen for humans. As Aspergillus spp. infections are becoming increasingly prevalent, their identification is necessary to investigate their characteristics and to select strategies for a correct management of these infections.

Author contribution

MZ, AFA and AV designed the research study, analysed the data and wrote the paper; EC and AP revised the paper. The authors thank Massimo Drosera, Carmela Sgro and Prof. Agostino Persi.

Conflict of interest

The authors declare no conflict of interests.

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Figure S1. Presence of fungal filaments at the direct microscopic examination of nail fragments in 30% potassium hydroxide.

Data S1. Materials and Methods.

Data S2. Supplementary References.

References

Dieckol, a phlorotannin of Ecklonia cava, suppresses IgE-mediated mast cell activation and passive cutaneous anaphylactic reaction

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Background

Mast cell activation mediated by immunoglobulin E (IgE) plays a crucial role in allergic diseases related to type I hypersensitivity including allergic rhinitis, asthma and atopic dermatitis (1, S1, S2). Binding of IgE to FcεRI receptors, followed by the cross-linkage of the IgE with the antigen, leads to the mast cell activation and secretion of preformed chemical mediators such as lipid mediators (leukotrienes and prostaglandins), granular contents ($\beta$-hexosaminidase and histamine), and inflammatory cytokines (interleukin [IL]-4, IL-6, IL-13, and tumor necrosis factor [TNF]-α), which initiates and exacerbates allergic inflammation (2,3, S3-6). Thus, stabilization of mast cells or blocking the released mediators’ action is a major strategy for the treatment of patients with type I hypersensitivity. While several agents such as non-steroidal anti-inflammatory drugs, steroids and immunosuppressants have been used, they more or less bring about adverse effects (4, S7). Therefore, safe candidates with both anti-allergic property and less toxicity have been expected.

Recent studies have suggested that phlorotannins, which were extracted from the brown seaweed Ecklonia cava, have an inhibitory effect on FcεRI expression on basophils and IgE-mediated granule release (5, S8). Another experiment determined a dieckol, one of the active ingredients from the extract, exerted potent anti-inflammatory effects by abrogating cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS) activation (6).

Questions addressed

Because there were no adverse effects when the dieckol was administered to mice (S9) and a chemical that possess a similar structure to dieckol suppressed basophil activation (5), we hypothesized that dieckol may also suppress mast cell activation in type I allergic responses. Therefore, we attempted to evaluate effects of dieckol on IgE-mediated activation of mast cells in vitro and experimental type I allergy in vivo.

Experimental design

Detailed methods are described in Data S1–S4. In in vitro experiments, bone marrow–derived cultured mast cells (BMCMCs) from male WBB6F,/+ mice and LAD2, a human mast cell line, were used to determine the release of $\beta$-hexosaminidase and histamine, or cytokine expressions. Flow cytometry analysis was also conducted to detect the expression of FcεRI and IgE-binding to the surface of BMCMCs and LAD2 cells. The in vivo effects of dieckol were analysed using a passive cutaneous anaphylactic (PCA) reaction model of mice induced by both anti-2,4-dinitrophenol (DNP)-IgE and DNP-BSA.

Results

Because mast cells play a critical role in type I allergic responses (2, S2, S4), we examined the effect of dieckol on degranulation of mast cells by measuring $\beta$-hexosaminidase and histamine, or cytokine expressions. Flow cytometry analysis was also conducted to detect the expression of FcεRI and IgE-binding to the surface of BMCMCs and LAD2 cells. The in vivo effects of dieckol were analysed using a passive cutaneous anaphylactic (PCA) reaction model of mice induced by both anti-2,4-dinitrophenol (DNP)-IgE and DNP-BSA.