Analysis of Chronic Biological Responses between host and fungus ball Using a Novel Aspergilloma Mouse Model

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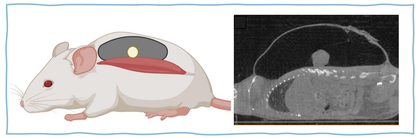
Purpose

The mechanism of the chronic biological responses against aspergilloma has been poorly understood due to the lack of appropriate animal models. Therefore, we attempted to create a new mouse model of aspergilloma and to analyze the chronic biological response of the host and aspergilloma over a period of several months using this model.

Methods

Aspergilloma mouse model

Live or heat-killed fungus ball which was prepared in vitro in advance was implanted in subcutaneous air-filled cavity created in the back of ICR mouse. Pre-immunization to *Aspergillus fumigatus* was performed in some mice by intraperitoneal administration of the homogenized solution of live fungus ball to mice for 1 month before implantation.



Histopathological and immunohistochemical analysis

Longitudinal histopathological and immunohistochemical examinations of the fungus ball and surrounding tissues was performed over a period of up to 5 months.

Galactomannan assay

We measured the concentration of galactomannan (GM) of fungus ball using the Bio-Rad Platelia GM assay to quantify the changes in fungal load over time.

In vitro cytotoxic analysis of *A. fumigatus* dead hyphae on macrophages

To determine the extent of host cell damage by *A. fumigatus* dead hyphae, the ^{51}Cr release assay was used.

Lipid Droplets Staining

The characteristics of the macrophages (RAW264.7 cell line) that phagocytosed the dead hyphae was examined with Oil-red-O stain in vitro.

Results

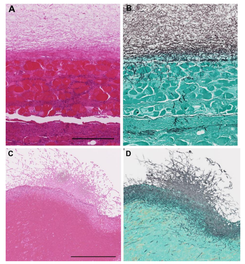


Figure 1. Histopathology of a non-immunized mouse on day 7 after live fungus ball implantation. H&E staining shows neutrophilic infiltration (A), and GMS staining shows hyphal invasion into the muscle layer (B). Tissue invasion is also observed in the histopathology of an immunized mouse on day 14 after live fungus ball implantation (C: H&E, D: GMS). Bars, 250 μm.

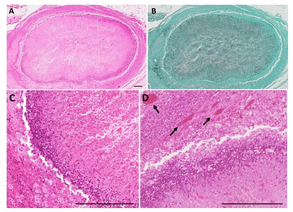


Figure 2. Histopathology of a mouse on 104 days after heat-killed fungus ball implantation. H&E staining (A) and GMS staining (B) are shown. Inflammatory cell infiltration inside and on the surface of the fungus ball (C) and fibrosis of the cavity wall (D) are seen. Black arrows indicate angiogenesis. Bars, 250 μm.

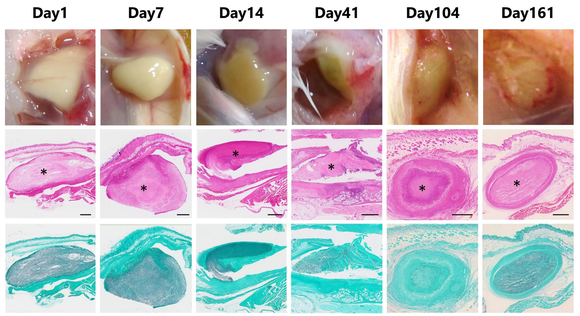


Figure 3. Temporal histopathology of day 1, 7, 14, 41, 104, 161 after implantation of dead fungus balls in mice. Visual photographs, H&E staining, and GMS staining for each specimen are shown in order from top to bottom. Asterisks indicate fungus balls. The heat-killed fungus balls had a whitish appearance until day 7, but after 14 and 41 days after implantation, they became yellowish brown with a purulent exudate around them. H&E staining shows inflammatory cell infiltration into the fungus ball through day41 and fibrous structure around the fungus ball after day 104, while GMS staining shows remaining hyphal mass even on day 161. Bars, 1 mm.

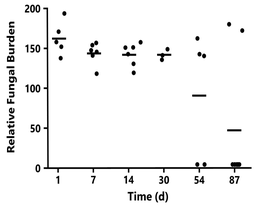


Figure 4. Time course of fungal burden measured from homogenized fungus ball solution. If the fungus ball is disappeared, it was counted as zero. Scores from each sample are plotted and the group mean is shown by a bar. GM was continuously detected in the fungus balls for 3 months, although the fungal burden gradually decreased.

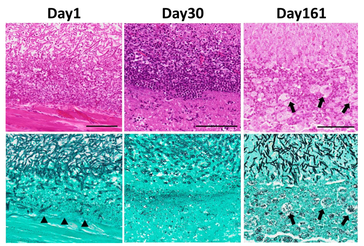


Figure 5. Detailed histopathology of the surface of the fungus ball on day 1, 30, 161. H&E staining shows the inflammatory cell infiltrate shifting from neutrophils to macrophages. GMS staining of the same area shows that the hyphae were broken into pieces by neutrophils on day 1 and phagocytosed by macrophages on day 161. Black arrowheads and black arrows indicate fragmented hyphae and swollen macrophages phagocytosing them, respectively. Bars, 100 μm.

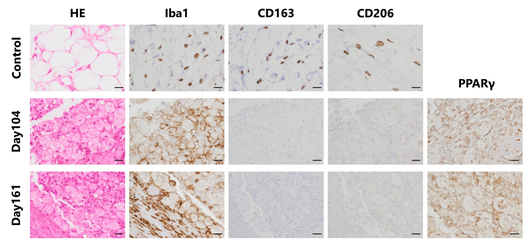


Figure 6. Control sections of uninfected skin and sections from the surface of fungus balls on day 104 and 161 were stained with macrophage-related (Iba1) and macrophage activation (CD163 and CD206) markers by immunohistochemistry (IHC). Iba1-positive cells were found in the skin, which were also positive for CD163 and CD206. In contrast, many Iba1-positive cells were found in the surface of the fungus ball on day 104 and 161, but the stains for both CD163 and CD206 were negative. The surface of the fungus ball was also stained for peroxisome proliferator-activated receptor gamma (PPAR-γ) protein, which is associated with lipid metabolism. The macrophages surrounding the fungus ball on day104 and 161 were positive for PPAR-γ. Bars, 20 μm.

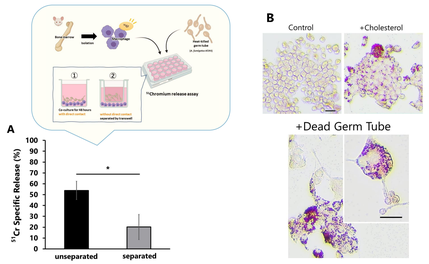


Figure 7. Impact of macrophages contact with heat-killed *A. fumigatus* germ tubes. (A) ^{51}Cr-release assay of macrophages and germ tubes with and without transwell separation. BMDM and germ tubes were plated with and without separation for 48 hrs. The results are the mean ±SD of 3 experiments, each performed in triplicate. *Statistically significant, p<0.01. (B) Oil-Red-O staining of RAW264.7 cells incubated with either cholesterol or dead *A. fumigatus* germ tubes. Control, the same staining was performed on RAW264.7 cells alone. RAW264.7 cells cultured with dead germ tubes were stained with Oil-red-O and showed high lipid loading similar to cells that had phagocytosed cholesterol . Bars, 20 μm.

Discussion

In this study, we developed a mouse model in which a fungus ball was implanted into an air-filled subcutaneous cavity. When a dead fungus ball was implanted, the model succeeded in reproducing many of the pathological findings of aspergilloma in humans. This result is consistent with reports of clinical pathology and fungal culture of aspergilloma which suggest that the fungus ball is mostly dead hyphae.[1,2] By analyzing the chronic immune response over a long period of time, we found that the inflammatory cell infiltration around the fungus balls shifted from neutrophils to macrophage-dominated cells. Furthermore, macrophages in direct contact with the dead hyphae were damaged and converted to foam cells. These findings suggest that the macrophages that ingest killed hyphal fragments become dysfunctional, making it difficult for them to eliminate the fungus ball. These results may be a major step forward in elucidating the previously unknown biological response to an aspergilloma.

Conclusion

We have characterized the crucial events that occur during the interaction of an A. fumigatus fungus ball in a cavity with the host using a novel mouse model. Further analysis of cytokines and gene expression of immune cells in and around the fungus ball which we are going to perform will help to elucidate the mechanisms of the immune response in more detail. Understanding the mechanism of macrophage transformation into foam cells may lead to the development of new therapeutic strategies for patients with aspergillomas. Because this mouse model is mini-mally invasive and simple, we believe that it may provide additional insights in the future by using genetically modified Aspergillus species and mice.

References

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2. Nomura K, et al. Int J Otolaryngol. 2013;2013: 731640.