Growth of Experimental Fungus Balls in the Pleural Cavity of Rabbits

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ABSTRACT. An attempt was made to produce an animal model of experimental aspergilloma by growing a fungus ball in the pleural cavity of the rabbit. Male white rabbits and the Aspergillus fumigatus PT-7 strain were used in this experiment. Instead of a cavity in the lung, a dead space was created in the pleural cavity using turpentine oil. Three weeks after creation of the cavity, an artificial fungus ball (about 10 mm in diameter) was inoculated into that space, and then hydrocortisone was injected into the same space. The size of the fungus ball in the pleural cavity of the rabbits receiving hydrocortisone increased gradually, its appearance was typically compatible with aspergilloma macroscopically and microscopically; e.g., coinciding with growth of the fungus in the cavity, there was infiltration of few cells into the central area of the fungus ball. Therefore, it was considered that experimental aspergilloma using the pleural cavity was successfully produced.

Key words: experimental aspergilloma — turpentine pleurisy — aspergillus fumigatus — hydrocortisone

Pulmonary aspergilloma is recognized as a complication of cavitating lung disease. Although there have been many reports describing pulmonary aspergilloma, it's pathogenesis has not been resolved completely. Therefore, an attempt was made to produce an animal model of aspergilloma. Instead of a cavity in the lung, a dead space was created in the right pleural cavity of animals using turpentine pleurisy and artificial fungus balls were inoculated into that space to produce aspergilloma in the pre-existing cavity. When one or ten artificial fungus balls were inoculated, they survived for 12 weeks in the pleural cavity, but their size did not increase[1)] with severe cell infiltration into the ball as in human cases. Therefore, an attempt was made using local immunosuppression to produce a model closer in similarity to human aspergilloma, which grows in the pleural cavity with mild cell infiltration.

MATERIALS AND METHODS

Male white rabbits weighing about 2 kg, and the Aspergillus fumigatus PT-7 strain (provided by the Pfizer Pharmaceutical Company Laboratory), isolated from a patient with pulmonary aspergilloma were used in this experiment. Artificial fungus balls, each about 10 mm in diameter and with a

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dry weight of about 21 mg, were prepared in vitro with the A. fumigatus PT-7 strain according to the method of Hisauchi.[2] Because recovery of simple pneumothorax might be easy, chemical pleuritis was created with turpentine oil to create an dead space (to maintain pneumothorax) in the right pleural cavity using the method of Sahn *et al.*[3] The animals were anesthetized by intravenous administration of 20 mg of sodium pentobarbital per kg of body weight.

Three weeks after the creation of chemical pleuritis, an artificial fungus ball was directly inoculated into the right pleural cavity of each rabbit.

After inoculation, hydrocortisone was injected into the same pleural cavity by the following two methods.

1) 12.5 mg/kg/day ×4 of hydrocortisone was injected into the right pleural cavity of eight rabbits. Two each were sacrificed at one, two, three, and four weeks after inoculation.

2) 1.25 mg/kg/day of hydrocortisone was injected into the right pleural cavity of six rabbits until they were killed. Two each were sacrificed at one, two, and four weeks after inoculation.

After thoracotomy, the pleura and lungs of each rabbit were observed macroscopically and microscopically. Isolation of the fungus from the lesions in the pleural cavity was performed. The double diffusion method (DD) and complement fixation method (CF) were used to detect serum antibody against aspergillus. Three commercial antigens (somatic antigen and culture antigen, Mercia Diagnostics, and scratch extract, Torii) were used with both methods.

RESULTS

When 12.5 mg/kg/day of hydrocortisone was administered four times;

The size of the fungus balls in the rabbits after each inoculation is shown in Fig. 1. Until the second week, the fungus balls grew and increased, but during the third and fourth weeks, they became smaller. In Fig. 2 the fungus

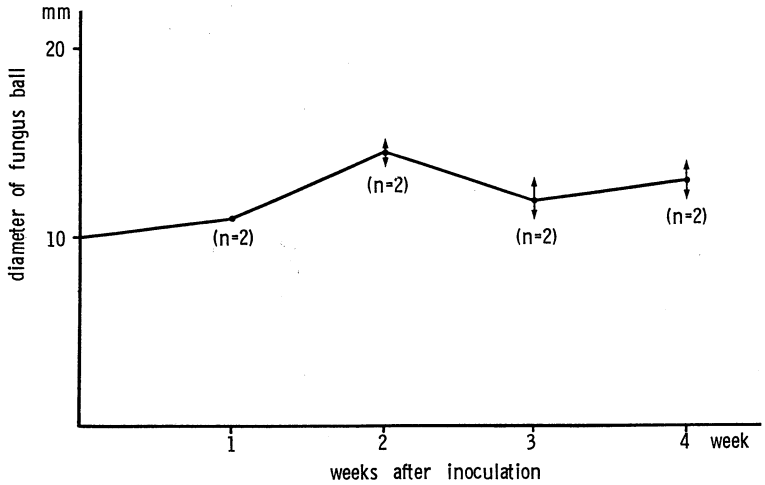

Fig. 1. The size of fungus ball in the rabbits received hydrocortisone (12.5 mg/kg/day ×4, locally) at each time after inoculation

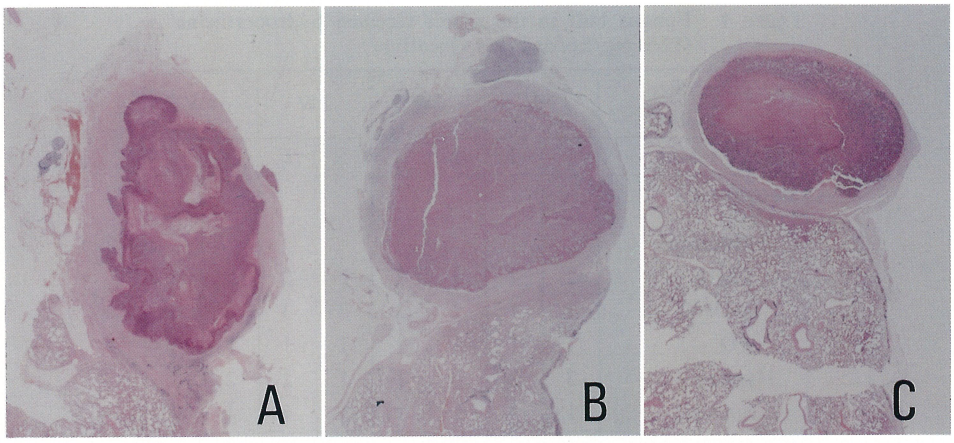

Fig. 2. Low magnification view (H.E.×3.0) at one (A), two (B), and four (C) weeks after inoculation (hydrocortisone 12.5 mg/kg/day ×4, locally)
Until two weeks after inoculation the size of fungus ball increased, but after three weeks they became smaller.

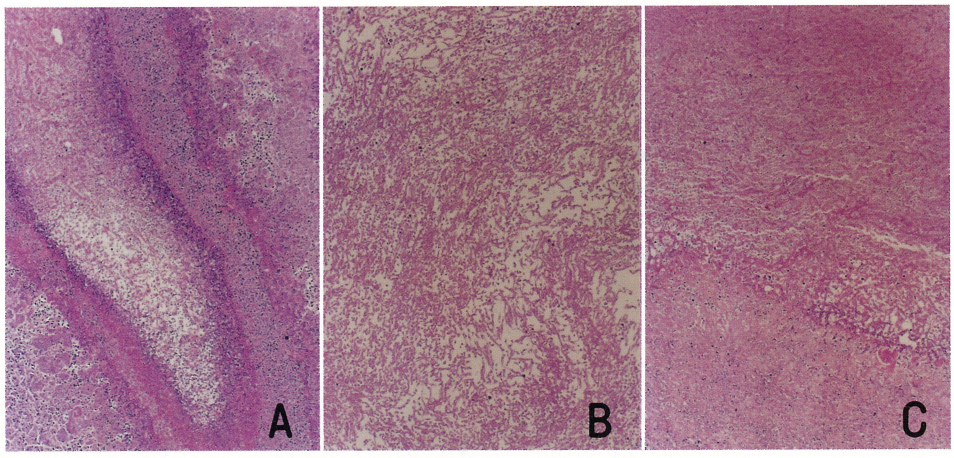

Fig. 3. Microscopic findings of fungus ball at one (A), two (B), and four (C) weeks after inoculation (hydrocortisone 12.5 mg/kg/day ×4, locally)
Cell infiltration was the weakest and the growth of hyphae was the highest at a week after inoculation. Thereafter cell infiltration increased and the growth of hyphae decreased gradually.

balls are shown under low magnification at one, two, and four weeks after inoculation. Microscopic findings indicated that cell infiltration was the weakest and the growth of hyphae was the highest at a week after inoculation. Thereafter cell infiltration increased and the growth of hyphae decreased gradually (Fig. 3). The degree of inflammatory cell invasion and the number of hyphae are shown in Table 1.

When 1.25 mg/kg/day of hydrocortisone was administered until the animals were killed;

Fig. 4 shows the size of the fungus balls after each inoculation. They grew

TABLE 1. Fungus ball in the rabbit received hydrocortisone (12.5 mg/kg/day ×4, locally)

Time after inoculation	1W		2W		3W		4W	
No. of rabbit	1	2	3	4	5	6	7	8
Fungus ball								
Cell infiltration								
PMN	2	2	2	2	2	2	1	1
MONO	1	1	2	2	3	3	3	3
EO	1	1	1	1	1	1	2	1
Hyphae	3	3	2	2	1	2	2	2
Necrosis	1	1	1	1	1	1	1	1
Pleura								
Thickness (mm)	0.8	0.8	0.8	0.8	0.8	0.9	0.8	0.9
Cell infiltration	2	2	3	3	3	3	3	3
Hyphae	0	0	0	0	0	0	0	0

1 : Mild, 2 : Moderate, 3 : Severe
PMN : Polymorphonuclear cell, MONO : Mononuclear cell
EO : Eosinophil

and increased with the passage of time. Low magnification views of fungus balls at one, two, and four weeks after inoculation are presented in Fig. 5. Microscopic findings showed that cell infiltration decreased and the number of hyphae increased in the fungus balls with the passage of time (Fig. 6, Table 2).

A fumigatus was isolated in all animals, but they were all negative for the antibody against A. fumigatus using the DD method and CF method.

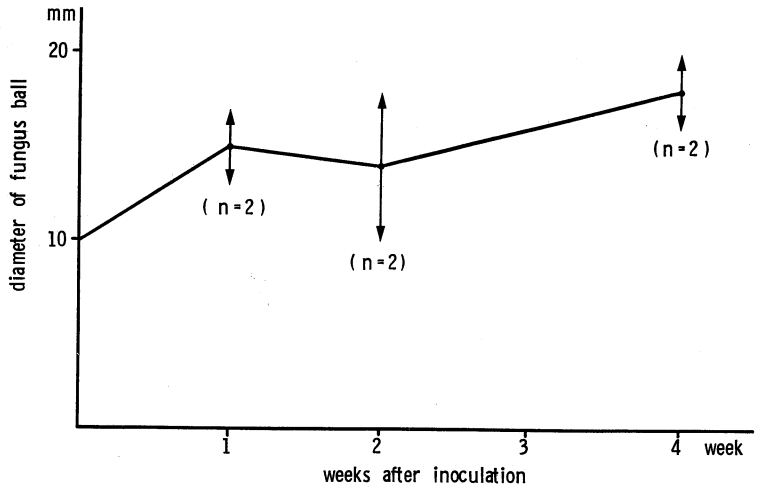

Fig. 4. The size of fungus ball in tne rabbits received hydrocortisone (1.25 mg/kg/day, every day, locally) at each time after inoculation

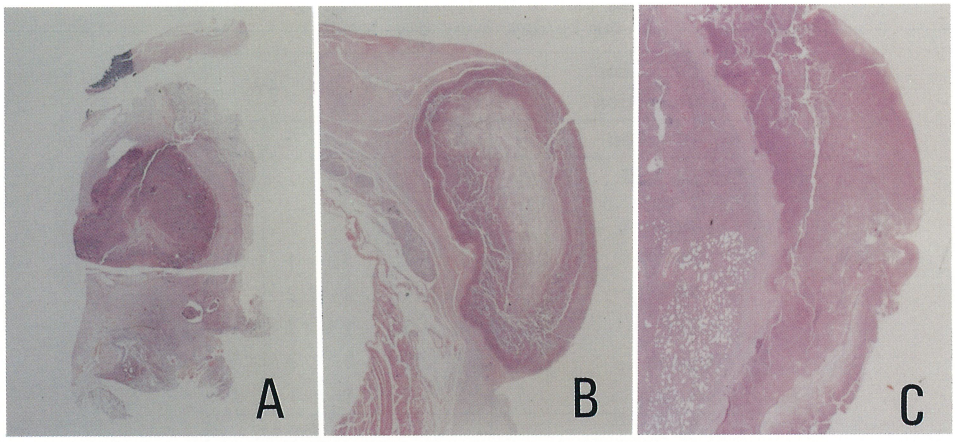

Fig. 5. Low magnification view (H.E. ×3.0) at one (A), two (B), four (C) weeks after inoculation (hydrocortisone 1.25 mg/kg/day, every day, locally)
The size of fungus ball increased with the passage of time.

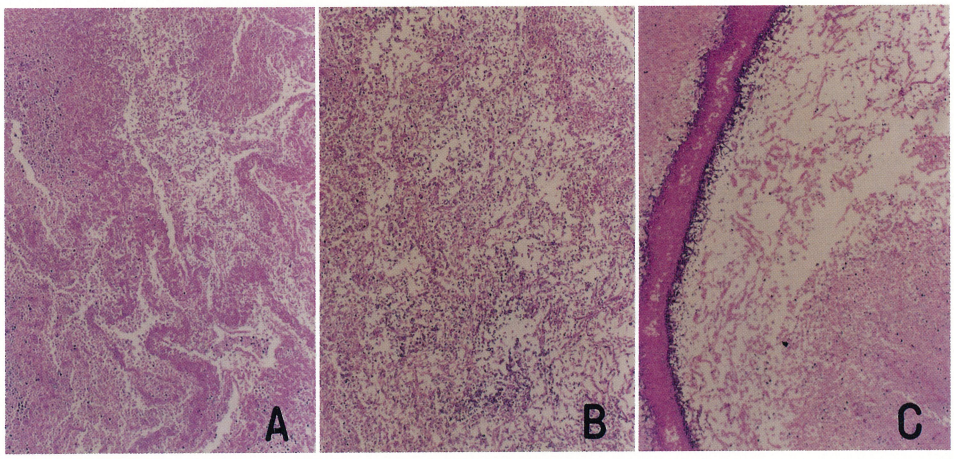

Fig. 6. Microscopic findings of fungus ball at one (A), two (B), and four (C) weeks after inoculation (hydrocortisone 1.25 mg/kg/day, every day, locally)
Cell infiltration decreased and the number of hyphae increased in the fungus balls with the passage of time.

DISSCUSSION

In several forms of pulmonary aspergillosis,[4)-6)] aspergilloma grows in an abnormal air space, either a lung cavity or a dilated bronchus, and produce a fungus ball. Fungus balls most frequently arise in association with chronic cavitary disease caused by tuberculosis oftenly.[7)-10)] Approximately 25 percent of patients have a history of the latter disease.[11)] In one long-term study 544 patients with healed open tuberculous cavities, 15 percent had typical appearance of aspergilloma or probable aspergilloma on chest X-ray with precipetins to aspergillus in their serum. At the end of a second survey, 20 percent of survivors had aspergilloma or probable aspergilloma.[12,13)]

TABLE 2. Fungus ball in the rabbit received hydrocortisone (1.25 mg/kg/day, every day, locally)

Time after inoculation	1W		2W		4W	
No. of rabbit	1	2	3	4	5	6
Fungus ball						
Cell infiltration						
PMN	2	2	2	2	1	1
MONO	1	1	1	2	2	2
EO	1	1	1	1	1	1
Hyphae	2	2	3	2	3	3
Necrosis	1	1	1	1	1	1
Pleura						
Thickness (mm)	0.3	0.3	0.3	0.3	0.3	0.3
Cell infiltration	3	3	3	2	2	1
Hyphae	0	0	0	0	0	0

1 : Mild, 2 : Moderate, 3 : Severe
PMN : Polymorphonuclear cell, MONO : Mononuclear cell
EO : Eosinophil

Several questions regarding the pathogenesis, immunoreaction, and treatment methods of pulmonary aspergilloma remain unanswered. I wished to produce an animal model of aspergilloma that would prove useful for finding answer to these questions.

Although many experimental studies of animal models of pulmonary aspergillosis have been reported,[14-21] a method for producing a saprophytic fungus ball has not yet been developed. Only Sawasaki *et al.*[22] have succeeded in producing animal models of aspergilloma, but their experimental technique is complicated and the aspergilloma differs from saprophytic type in human cases. Therefore, an attempt was made to produce and animal model of experimental aspergilloma by a method different from those described previously. A dead space was created in the pleural cavity using turpentine pleurisy,[3] and this space was used instead of a pre-existing cavity in the lung to produce a fungus ball. In addition, I made the artificial fungus balls used in this experiment.[2] Most cases of aspergilloma are thought to arise from colonization and proliferation of the fungus in a pre-existing pulmonary cavity.[23,24] The histopathologic characteristics of aspergilloma disclose an intracavitary mass of tangled mycelia, fibrin, mucus, amorphous debris, inflammatory cells, degenerating blood, and epithelial elements.[23,25-27] In our previous experiment, when one or ten artificial fungus balls were inoculated into the pleural cavity of the rabbits, the fungus balls did not increase in size over a 12-weeks period. Although hyphae existing in the fungus balls grew and increased gradually, the fungus balls were not completely compatible with those in human cases; e.g., they did not increase in size.[1] Therefore, local hydrocortisone was employed in this experiment with the expectation that the fungus balls would increase with the passage of time, since hydrocortisone impairs the defense system (mainly macrophages and polymorphonuclear cell)

in host to A. fumigatus.[28-30] And I think effect of hydrocortisone made negative for antibody in DD and CF method. Again, as fungus ball in rabbit received hydrocortisone locally grew and increased compared with those in rabbits not receiving hydrocortisone. Microscopically, there were a few infiltrative cells in the central area of the fungus balls, as a result of which they bore a closer similarity to those in human cases than did fungus balls in our previous experiment.[27] Although my experimental fungus ball does not exist in the lung but in the pleural cavity, I consider that an experimental animal model of aspergilloma has been successfully produced using my new-method. I believe this experimental model should be useful for study of the pathogenesis, immunoreaction, and treatment method of clinical aspergilloma.

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