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Case Report: Pathomorphology of an experimental disseminated *Aspergillus fumigatus* infection in rabbits

Fallbericht: Pathomorphologie einer experimentellen disseminierten *Aspergillus fumigatus*-Infektion bei Kaninchen

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Summary: In following a formerly successful protocol designed to produce antibodies to *A. fumigatus* (Fres.) we observed a disseminated, lethal fungal infection in healthy, specific pathogen-free (SPF) rabbits. The pathomorphological findings included multiple miliary to avenaceous whitish nodules in the livers and kidneys, mycotic mesencephalitis, nephritis, hepatitis, myocarditis, hemorrhagic enteritis, and splenitis. The hyphae were surrounded by necrosis, which also occurred in the liver without the hyphae. Comparative gas chromatographic and metabolic investigations on this strain and some environmental *A. fumigatus* strains showed significant differences. The findings are discussed with particular reference to the pathogenicity of *A. fumigatus*.

Zusammenfassung: Ein von uns mehrfach erfolgreich eingesetztes Protokoll zur Gewinnung von *A. fumigatus*-Antikörpern führte zu einer disseminierten letalen Aspergillose bei spezifisch pathogenfreien Kaninchen. Die pathomorphologischen Befunde umfaßten multiple miliare bis haferkorngroße, grau-weiße Herde in der Leber und Nieren, mykotische Mesenzephalitis, Nephritis, Hepatitis, Myokarditis, hämorrhagische Enteritis und Splenitis. Die Hyphen waren von Nekroseherden umgeben. Letztere waren in der Leber auch ohne Hyphen, trotz Serienschnitte zu beobachten. Vergleichende gas-chromatographische und metabolische Untersuchungen des eingesetzten Stammes mit anderen Umweltisolaten von *A. fumigatus* zeigten signifikante Unterschiede. Die Befunde wurden unter besonderer Berücksichtigung der Pathogenität von *A. fumigatus* diskutiert.

Introduction

Aspergillus fumigatus is an ubiquitous mould occurring commonly on decaying leaves and wood, compost, feed and air dust (Kane and Mullins, 1973; Solomon and Burge, 1975; Piens et al., 1993; Millner et al., 1980; Ellis, 1980; Ogundero, 1980; Jones and Cookson, 1983; Mullins et al., 1984; Abdel-Hafez et al., 1986). Because disseminated *A. fumigatus* infections are often observed in association with underlying diseases such as AIDS (Bonatz et al., 1991; Sigh and Rihs, 1991; Roux et al., 1992), malignant cancers (Niimi et al., 1991; Cohen and Heffner, 1992), stress situations (Lucet,1897; El-Kouly et al., 1992), or involving immunosuppression (Rippon and Anderson, 1978; Solomon and Burge, 1975; Jensen et al., 1991; Rabin; 1992), the fungus is called an „opportunistic" pathogen. Reports of primary disseminated *A. fumigatus* infections are very limited, and in most cases an exact statement about the immunologic status of the patients is not possible on account of the spontaneous nature of the infection (Lucet, 1897; Roux et al., 1992; Korbel et al., 1993).

We report here a case of disseminated *A. fumigatus* infection in SPF rabbits, describe its pathomorphological lesions, and present the results of a comparative study of the subjet strain with environmental strains.

Case history

In order to produce *A. fumigatus* antibodies we inoculated each of two healthy, female SPF rabbits (case 1 & case 2; race: Flemish giant) intravenously with 10^6 live *A. fumigatus* (Fres.) conidia (strain # 30A) according to a procedure previously described (Mohan et al., 1980). This procedure was formely successful in our laboratory for development of antibody used in common Fluorescent Antibody reactions. The strain was isolated 28 years ago from human aspergillosis.

On the morning of the sixth day *post inoculation,* one animal (case 1) showed inappetence, apathy, and a bending of its head to the left. Skittishness, torticollis, and circular movements were observed. In the afternoon the animal lay crumpled in its cage. At 3:00 p.m. the 2nd animal (case 2) showed light clinical signs similar to those observed in the first (case 1). Following the recommendations of the Institutional Animal Care And Use Committee, the first rabbit (case 1) was euthanised the same day. The second (case 2) died overnight. We examined both rabbits pathologico-anatomically, histologically, immunohistologically, and microbiologically.

Other, non-infected animals, raised in the same pen remained healthy. There was no evidence for concurrent viral or bacterial infections. We studied the *A. fumigatus* strain used in the present investigation, and 6 environmental *A. fumigatus* strains, isolated from biowaste, compost and chicken litter, for their ability to assimilate various C- and N-compounds. The strains were also examined by gas chromatography according to the procedure previously described (Szepesy, 1970). These investigations were done in triplicate.

Results

The autopsy findings in both animals were nearly identical. Multiple miliary to avenaceous, whitish nodules were present in the liver and kidney in both cases, and in the cecal serosa in case 2.

Histologically, hyphae were seen in all organs, but with varying intensities.The kidney showed extensive tubulonephritis and the hyphae often appeared in stellate formations (Fig. 1). The hyphae were included in a cell detritus and surrounded by neutrophils and

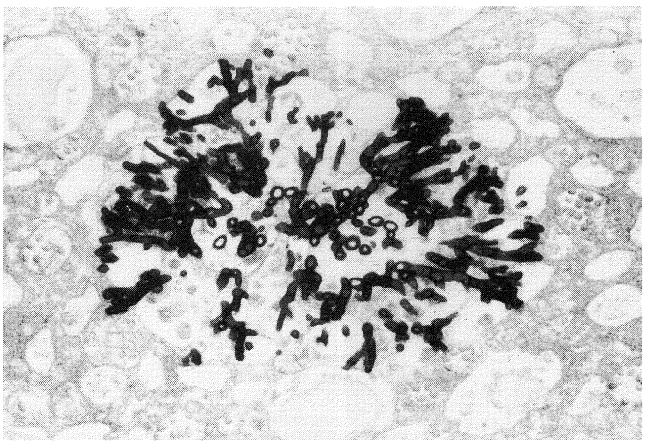

Fig. 1 Kidney. Note the stellate, centrifugal radiating hyphae surrounded by necrotic focus. (PAS-Stain, ×400)

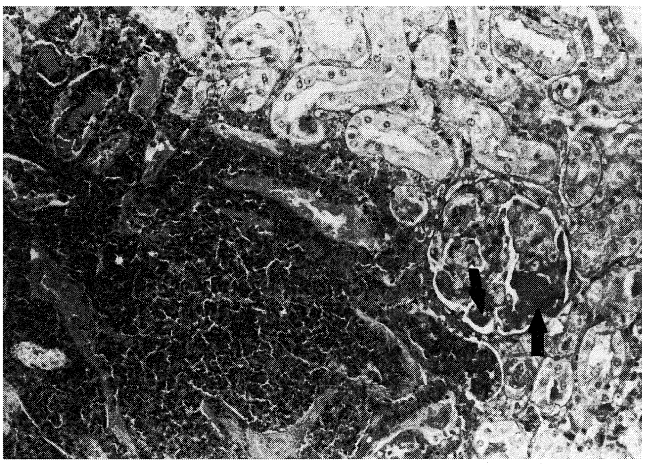

Fig. 2 Nephritis mycotica with capillary thrombosis in the glomerulum (arrows). (PAS-Stain, ×200)

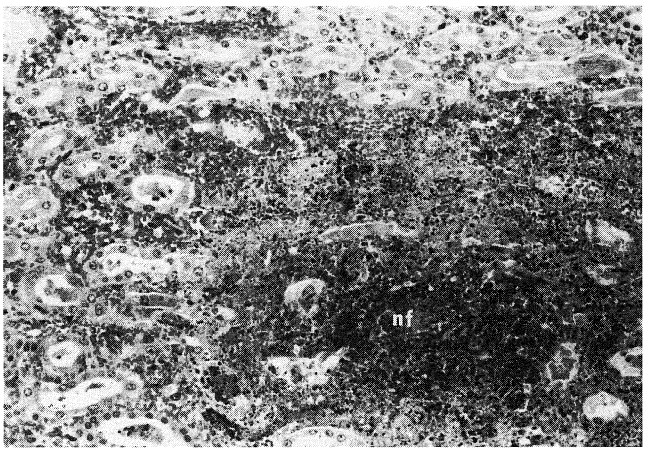

Fig. 3 Liver with interlobulary necrotic focus (nf.). (H. & E.-Stain,

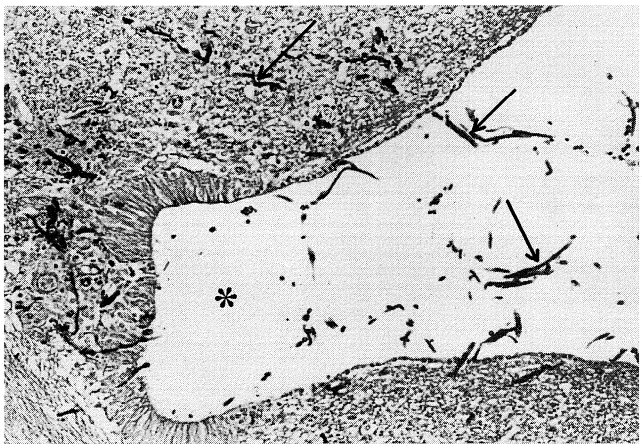

Fig. 4 Mesencephalitis mycotica (* = 3rd ventricle; arrows show hyphae, GMS-Stain, ×400)

eosinophils. The glomeruli were almost always intact, although they were inside or close to necrotic foci. Some glomeruli were damaged *per diabrosin* (Fig. 2) and the glomerular capillaries showed thrombosis. Rare hyphae were observed at the Bowman's capsules and cell accumulations in the Bowman's space. Intertubulary inflammatory hyperemia and dichotomously branching hyphae were particularly noted in the renal medulla.

The lesions in the liver were multifocal, without consideration of the hepatic architecture. While the constellations of the cell detritus, hyphae, and foci of necrosis were similar to those in the kidney, more necrosis of hepatocyte groups was detected without the presence of the etiologic agent (Fig. 3). Some foreign body giant cells were present in the cell detritus.

The brain, particularly the mesencephalon, showed focal necrotizing processes beginning from the 3rd ventricle (Fig. 4).

The lesions in the spleen, lymph nodes, lungs, and myocardium were not extensive. While the lesions were concomitant with necrosis in the spleen, lymph nodes, and myocardium, the agent was seen in the lung interstitium causing a histiocytic accumulation.

An extensive hemorrhagic typhlitis was seen. Foreign body giant cells were noted also in the intestine. The hyphae extended from the submucosa to the top of the villi, concomitant with an accumulation of histiocytes, neutrophils, and eosinophils.

A. fumigatus was isolated from all organs by standard microbiological methods.

Physiologic Activities: None of the strains was able to assimilate urea, nitrate, or lactose. However the rabbit strain assimilated sucrose and trehalose after 20 h incubation at 37 °C, and 48 h incubation at 25 °C, faster than the environmental strains did. Under the same conditions, trehalose was assimilated by 4 environmental strains (# 91301, 88602, 84013 and 36003) after 48 to 72 h. On Blood agar strain 30A showed a 2 cm larger hemolytic radius at 42 °C than did the other strains. Gas chromatography revealed, that the environmental strains were quantitativly and qualitativly incapable of producing a large number of compounds (Fig. 5).

Discussion

Lucet in 1897 attributed a pyrogenic effect to metabolic products of *A. fumigatus*. Recent investigations have revealed that the fungal toxins are also nephrotoxic, dermonecrotic, hemolytic, and cytotoxic (Spreadbury et al., 1989; Rau et al., 1961; Tilden et al., 1961; Korbel et al., 1993), and inhibit ribosomal translation (Lamy et al., 1991). Not only does the mechanical destruction by the hyphae have a pathogenetic importance, but also the damages caused by the toxins. These damages (e.g., necrosis without the presence of hyphae) were particularly seen in the liver in this study. In contrast to reports of other investigators (Lucet, 1897; Zook and Migaki, 1985; El-Kouly et al., 1992) and in spite of the hemorrhagic enteritis, we observed no diarrheal signs in the animals.

Death of the animals is attributed to disseminated *A. fumigatus* infection, but the unexpected lethality could presumedly have been influenced by due to physiological activities which

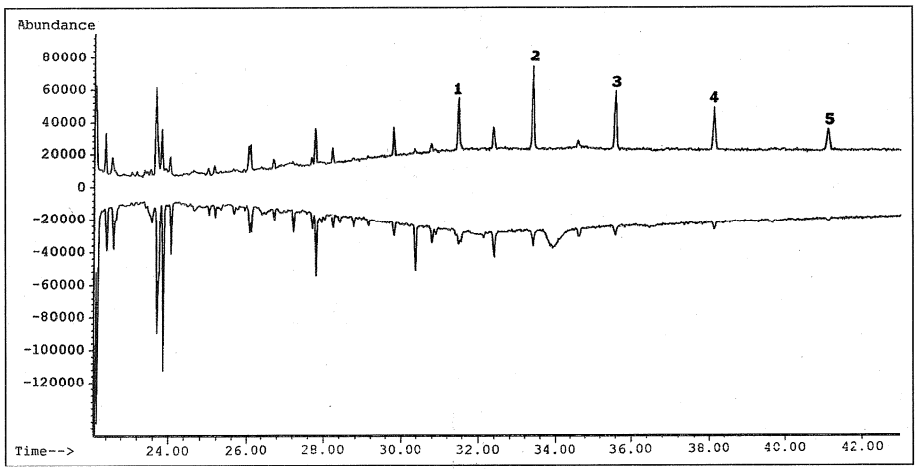

Fig. 5 Gas chromatograms of the strain # 30A (above) and an environmental strain (# + 91301) (below) 1 = Hexacosan, 2 = Heptacosan, 3 = Octacosan, 4 = Nonacosan, 5 = Triacontan

were absent in the environmental strains (e. g. rapid assimilation of sucrose and trehalose, hemolysis and production of various C-compounds, detected by the gas chromatography). Inoculation with raising of antibody had been performed without lethal or pathogenic effects 11 years earlier under identical conditions, including culture and rabbit strain, inoculum concentration, and rabbit weight (Gordon, pers. observation). Pathologico-anatomical findings are indistinguishable from those of other septicemic infections (e.g., salmonellosis). Only the histology, particularly with the GMS stain, reveals the fungal etiology.

Rippon et al (1971) found significant differences between soil and human isolates of *A. terreus* in regard to virulence, and *in vitro* growth rate. An intravenous inoculum of 10^4 spores of human isolates in mice led to exitus lethalis, whereas 10^7 to 10^8 of soil isolates were required for the same results (Rippon et al., 1971).

These investigations demonstrate that *A. fumigatus*, depending on the strain, can be lethally pathogenic even without underlying diseases and predisposing factors.

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