MYCOLOGY

Experimental pathogenicity of four opportunist *Fusarium* species in a murine model

E. MAYAYO, I. PUJOL* and J. GUARRO†

Servei Patologia, Hospital Universitari de Tarragona Joan XXIII, *Laboratori de Microbiologia, Hospital Universitari de Sant Joan de Reus and †Unitat de Microbiologia, Facultat de Medicina, Universitat Rovira i Virgili, 43201 Reus, Tarragona, Spain

A murine model with immunocompetent animals was used in a comparative study of experimental pathogenicity of 13 isolates belonging to the four most frequent pathogenic species of *Fusarium* in man (*F. solani*, *F. oxysporum*, *F. verticillioides* and *F. proliferatum*). Inocula of 5×10^6 conidia/mouse of each isolate of *Fusarium* were injected into a lateral vein of the tail of the mice to produce a systemic infection. *F. solani* was the most virulent species; the five strains of this species assayed caused the death of all the animals tested in <19 days. The other species of *Fusarium* were not virulent in this model. The organs mainly affected by *F. solani* were the kidneys and the heart. These findings correlate with the clinical evidence and demonstrate that there is a high risk associated with infection by *F. solani*, especially for immunocompromised patients.

Introduction

In recent years, filamentous fungi have been implicated in an increasing number of opportunist infections in immunocompromised patients [1]. The genus *Fusarium* is particularly important and *F. solani* is the species that most frequently causes severe disseminated infections in man. Other infectious species, encountered to a lesser extent, are *F. oxysporum* and *F. verticillioides*. *F. proliferatum* has also caused a small number of cases. These infections have a high mortality and as yet there is no effective treatment against them. The in-vitro susceptibility of *Fusarium* spp. to the available antifungal drugs does not allow the prediction of any adequate treatment [2]. The pathogenicity of *F. solani* in mice has also been clearly demonstrated [3, 4]. Nevertheless, why this species is the most frequent in these processes is unknown. It is probably the most prevalent or the most virulent, or both, and the infections produced by other species are more easily controlled by the host defences and are not disseminated.

This report describes a comparative study of the pathogenicity of the four most important species of *Fusarium* in human diseases in a murine model [3].

Materials and methods

Test isolates

Thirteen isolates of *Fusarium* spp. (10 from clinical sources and 3 from environmental sources) were used (Table 1): *F. solani* (5), *F. oxysporum* (5), *F. verticillioides* (2) and *F. proliferatum* (1). The fungi were stored on potato carrot agar (PCA) slants covered by mineral oil and as a suspension in water at room temperature.

Preparation of inoculum

Strains were subcultured on to PCA slants and incubated at 30°C for 15 days. The inoculum was prepared by flooding the surface of the agar slant with distilled water, scraping the sporulating mycelium with a teasing needle and drawing up the resultant suspensions with a sterile pasteur pipette. The suspensions were then filtered once through sterile gauze to remove hyphae and a suspension with >90% conidia was obtained. The conidial suspensions were counted with a haemocytometer and counts were verified by serial dilutions on PCA plates which were incubated at 30°C for 72 h. The suspensions were diluted in sterile NaCl 0.9% to produce the final inoculum of 2.5×10^7 conidia/ml.

Animals

OF₁ male mice (Griffa, Barcelona, Spain) weighing 30 g were used. Mice were housed 10/box in standard

Received 12 Dec. 1997; revised version received 27 July 1998; accepted 29 July 1998.

Corresponding author: Professor J. Guarro (e-mail: umb@fmcs.urv.es).

Table 1. Isolates of *Fusarium* studied and survival of immunocompetent mice within 30 days

			Survival (days)	
Strain		Origin	Median	Range
<i>F. solani</i>	FMR 4927	Skin	7	2–19
<i>F. solani</i>	FMR 4928	Subcutaneous abscess	2	2–16
<i>F. solani</i>	FMR 4931	Skin	9	1–13
<i>F. solani</i>	FMR 5207	Skin	2	1–14
<i>F. solani</i>	91-798	Blood	14	11–18
<i>F. oxysporum</i>	FMR 5203	Blood	>30	1–>30
<i>F. oxysporum</i>	7526/V	Nail	>30	>30
<i>F. oxysporum</i>	117-F	Skin	>30	>30
<i>F. oxysporum</i>	CBS 620.87	Kernel of <i>Hordeum vulgare</i>	>30	>30
<i>F. oxysporum</i>	FMR 4396	Soil	>30	2–>30
<i>F. verticillioides</i>	91-1478	Blood	>30	>30
<i>F. verticillioides</i>	FMR 4393	Soil	>30	3–>30
<i>F. proliferatum</i>	90-1007	Lung	>30	>30

FMR, Faculty of Medicine of Reus.

boxes with corn-cob bedding and given food and water *ad libitum*.

Infection model

Inoculum suspensions (200 µl volumes of 5×10^6 conidia/mouse) of each fungal strain were injected into the lateral tail veins of groups of 20 mice. Mortality was recorded daily for 30 days after inoculation.

Histopathology

Representative formalin-fixed portions of kidneys, liver, spleen, lungs, eyes, heart and brain obtained from animals at autopsy were stained with haematoxylin and eosin, PAS and Grocott (methenamine silver).

Results

Table 1 summarises the results obtained from inoculating immunocompetent mice with four species of *Fusarium* spp. *F. solani* was the most virulent species; the five strains tested caused the death of all the animals within the study period (no animal survived longer than 19 days). The other three species caused a significantly lower degree of mortality varying from 0 to 40% of the animals tested. Three strains of *F. oxysporum*, one of *F. verticillioides* and the single strain of *F. proliferatum* did not cause the death of any animal.

The distribution of affected organs in mice infected by *F. solani* is shown in Table 2. The organs that were mainly affected were the kidneys (in all the animals tested) and the heart (in c. 50% of the animals tested). These organs showed lesions made up of necrotic material with polymorphonuclear cells and variable amounts of branched, non-pigmented hyphae. Angio-invasion and thrombosis were also observed frequently. Granulomatous inflammation was never observed in the tissue sections of the infected organs.

Kidneys were always affected bilaterally with similar lesions in each organ. The changes induced by the five strains of *F. solani* tested were progressive. The parenchyma was affected in proportion to the duration of the fungal infections. The infectious fungal elements were observed, in some glomeruli and tubules of the cortical zone and with practically no inflammatory response, between 1 and 2 days after inoculation. After 4 days, a high degree of infection was noticed with the presence of micro-abscesses, a certain amount of parenchymal destruction and a greater abundance of hyphae. The inflammatory response was weak. After 7 days, the presence of fungal elements was more evident at the level of the pelvis and the parenchymatous foci disappeared without granuloma being detected. In those mice that survived longer than 11 days, both kidney pelvises were dramatically invaded by hyphae; they were clearly dilated and macroscopically white. An inflammatory response surrounding the affected area was

Table 2. Distribution of affected organs in mice infected by *F. solani* that died during the experiment

Strain no.	Number of animals tested	Number of animals affected						
		Kidneys	Lungs	Heart	Brain	Eyes	Liver	Spleen
FMR 4927	20	20	6	12	10	8	4	0
FMR 4928	20	20	10	12	4	4	2	2
FMR 4931	20	20	8	12	4	8	4	4
FMR 5207	20	20	4	10	4	6	2	0
91-798	20	20	5	10	4	8	2	2

FMR, Faculty of Medicine of Reus.

observed. There was also a considerable degree of myocardial involvement which was progressive at first, but which soon decreased and finally almost disappeared. The fungus invasion of the myocardium suddenly decreased at about the same time as a massive involvement of the renal pelvis was observed.

In decreasing order the other organs affected were the lungs, eyes, brains, liver and spleen, but in these cases the number of animals affected was closely related to the strain. Visible skin lesions were not seen.

Discussion

The murine model described by Legrand *et al.* [3] has proved to be very useful in comparing the pathogenicity of the most frequent species of *Fusarium* causing fungal infection in man. The four species tested (not always easily differentiated from each other when derived from clinical sources [2]) showed different degrees of pathogenicity. In the present study, *F. solani* was practically the only species that was highly virulent in immunocompetent mice. Although the five strains tested caused the death of all animals, variable pathogenicity over the period of the experiment was observed among them; the clinical isolate from a blood culture, with a survival median of 2 weeks, was the least virulent. The finding that *F. solani* was more pathogenic than the other species in this model is in agreement with the clinical evidence, because most disseminated fusarial infections are caused by *F. solani*.

In a recent report on disseminated *Fusarium* infections it was noted that 27 of the 83 cases reported were caused by *F. solani*, 12 by *F. verticillioides* and 11 by *F. oxysporum* [2]. Unfortunately, almost all of the strains belonging to these three species were resistant *in vitro* to all the antifungals tested [5]. However, *F. solani* strains were most resistant to amphotericin B, the antifungal agent most widely used in these infections. The mean MIC of amphotericin B against *F. solani* in a microdilution method was 3.47 mg/L, while those of *F. oxysporum* and *F. verticillioides* were 2.13 mg/L and 1.98 mg/L, respectively [5]. Similarly, the efficacy of the treatment regimens assessed *in vivo* is questionable. Of greater importance in immunosuppressed patients is the recovery of the neutrophil count, which appears to be essential for clinical resolution. In only one of the 27 cases of resolved *F.* infection did the neutrophil count not recover [6]. This was the only case in which a patient survived disseminated infection despite severe immunosuppression and persistent granulocytopenia. The patient underwent a bone marrow transplant and received amphotericin B, rifampicin and granulocyte transfusions. However, in this case the causative agent was *F. proliferatum*, a species that did not cause the death of any animal in the present study. In another recent case of fungaemia caused by a *Fusarium* sp. the

infection responded to the oral triazole SCH 39304, despite progressive neutropenia, but in this case the species was *F. oxysporum* [7], which was also less virulent than *F. solani* in the present study. A recently reported infection caused by *F. oxysporum* in an HIV-infected patient with a port-a-cath was treated successfully with liposomal amphotericin B [8].

In all the animals infected by *F. solani*, the kidneys and heart were the most frequently affected organs. This was also observed by Legrand *et al.* [3] and it is in agreement with the results of previous studies where other fungi were assayed – such as *Aphanascus* spp., the causative agents of rare superficial infections in man [9] and *Scedosporium prolificans*, an emerging agent of severe invasive infections [10]. However, brain involvement was noticed with the latter fungus. Involvement of the brain and eye (as much in intra-ocular structures as in neighbouring muscles) was also observed in the present study. Keratitis is a common type of infection caused by *F. solani* in immunocompetent individuals. It is relatively frequent and is distributed worldwide [11–17]. It mainly follows trauma, nail scratches, ocular surgery or complications caused by contact lens wear [18]. In neutropenic patients with disseminated fusarial infections, skin lesions are found in about 75% of individuals [2]. However, no visible cutaneous manifestations were observed in the present study. This is probably explained by the fact that the test animals were not immunocompromised. The experimental study performed by Legrand *et al.* [3] did not report skin lesions in neutropenic mice.

We thank Dr F. Odds (Janssen Research Foundation) for critical review of this manuscript and Drs Alonso, Ballester and Rinaldi, and the Curator of CBS (the Netherlands) for supplying strains for the study.

References

- Guarro J, de Hoog GS, Figueras MJ, Gené J. Rare opportunistic fungi. In: de Hoog GS, Guarro J (eds) Atlas of clinical fungi. Baarn, Centraalbureau voor Schimmelcultures. 1995: 241–675.
- Guarro J, Gené J. Opportunistic fusarial infections in humans. *Eur J Clin Microbiol Infect Dis* 1995; **14**: 741–754.
- Legrand C, Anaissie E, Hashem R, Nelson P, Bodey GP, Ro J. Experimental fusarial hyalohyphomycosis in a murine model. *J Infect Dis* 1991; **164**: 944–948.
- Anaissie EJ, Hachem R, Legrand C, Legenne P, Nelson P, Bodey GP. Lack of activity of amphotericin B in systemic murine fusarial infection. *J Infect Dis* 1992; **165**: 1155–1157.
- Pujol I, Guarro J, Gené J, Sala J. In-vitro antifungal susceptibility of clinical and environmental *Fusarium* spp. strains. *J Antimicrob Chemother* 1997; **39**: 163–167.
- Barrios NJ, Kirkpatrick DV, Murciano A, Stine K, Van Dyke RB, Humbert JR. Successful treatment of disseminated *Fusarium* infection in an immunocompromised child. *Am J Pediatr Hematol Oncol* 1990; **12**: 319–324.
- Raad I, Hachem R. Treatment of central venous catheter-related fungemia due to *Fusarium oxysporum*. *Clin Infect Dis* 1995; **20**: 709–711.
- Eljaschewitsch J, Sandfort J, Tintelnot K, Horbach I, Ruf B. Port-a-cath-related *Fusarium oxysporum* infection in an HIV-infected patient: treatment with liposomal amphotericin B. *Mycoses* 1996; **39**: 115–119.
- Cano J, Mayayo E, Guarro J. Experimental pathogenicity of

- Aphanoascus* spp. *Mycoses* 1990; **33**: 41–45.
10. Cano J, Guarro J, Mayayo E, Fernández-Ballart J. Experimental infection with *Scedosporium inflatum*. *J Med Vet Mycol* 1992; **30**: 413–420.
11. Foster CS. Fungal keratitis. *Infect Dis Clin North Am* 1992; **6**: 851–857.
12. Liesegang TJ, Forster RK. Spectrum of microbial keratitis in South Florida. *Am J Ophthalmol* 1980; **90**: 38–47.
13. Forster RK, Rebell G. The diagnosis and management of keratomycosis. I. Cause and diagnosis. *Arch Ophthalmol* 1975; **93**: 975–978.
14. Gugnani HC, Talwar RS, Njsoku-Obi ANV, Kodilinye HC. Mycotic keratitis in Nigeria. A study of 21 cases. *Br J Ophthalmol* 1976; **60**: 607–613.
15. Ishibashi Y. [Keratomycosis in Japan reported from 1976 to 1980.] *Nippon Ganka Gakkai Zasshi* 1982; **86**: 651–656.
16. Jones DB, Sexton RR, Rebell G. Mycotic keratitis in south Florida, a review of 39 cases. *Trans Ophthalmol Soc UK* 1969; **89**: 781–797.
17. Polack FM, Kaufman HE, Newmark E. Keratomycosis. Medical and surgical treatment. *Arch Ophthalmol* 1971; **85**: 410–416.
18. Strelow SA, Kent HD, Eagle RC, Cohen EJ. A case of contact lens related *Fusarium solani* keratitis. *CLA Op J* 1992; **18**: 125–127.