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Comparison of the virulence of *Scedosporium prolificans* strains from different origins in a murine model

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***Scedosporium prolificans* is an emerging opportunist fungus that causes different types of infections in immunocompetent and immunosuppressed people. These infections show an irregular geographical distribution and, generally, disseminated systemic infections are noticed only in specific countries. This study used a murine model of disseminated infection by this fungus to assess if strains from different origins have different virulence. Two strains from each of four different sources (disseminated infection, localised infection, asymptomatic cystic fibrosis patients and the environment) were tested. Two strains of *S. apiospermum* of clinical origin were also included in the study; these were clearly less virulent than those of *S. prolificans*. The *S. prolificans* strains tested were classified in three groups according to their virulence. The groups with higher and lower virulence were represented by only one strain each, and the intermediate group contained six strains. No significant differences were found between the strains from different geographic areas or different forms of disease.**

Introduction

Scedosporium prolificans is a recently emerging opportunist fungus that causes different degrees of infection depending on the route of infection and the immune status of the host [1–3]. In approximately a quarter of the published cases, the isolation of the fungus is of doubtful clinical significance, being mainly from pulmonary colonisation in cystic fibrosis patients [1, 4, 5]. When *S. prolificans* infects immunocompetent people, there has usually been prior trauma in the form of puncture wounds, skin ulcers or surgery, and it produces localised infections that involve skin, bone or joints. In immunocompromised patients and especially in those suffering haematological malignancies, lesions spread and they are usually fatal in less than a month [6]. An altered immune status of the host seems to be the main cause of invasion by this mould, which mimics most of the pathogenic aspects of other opportunist fungi such as *Fusarium* spp. [7], *Acremonium* spp. [8] or even *Aspergillus fumigatus* [9]. In these cases the infection is probably acquired by inhalation of conidia and the fungus is able to develop in practically any body organ, although it mainly does so in kidney, lung, brain, spleen, thyroid and myocardium [1]. *S. prolificans* shows universal in-vitro and in-vivo resistance to available antifungal drugs [3, 10, 11], and recovery from neutropenia has been considered a mandatory prerequisite for resolving the infection irrespective of the antifungal treatment used [3].

The pattern of infections produced by *S. prolificans* has changed since the early reports. In the 1980s they were usually localised infections that involved musculoskeletal tissues in immunocompetent patients [12–14], but since 1990 disseminated infections have increased dramatically, mainly among patients with haematological malignancies, with a mortality rate close to 100%.

Infections by *S. prolificans* are not homogeneously distributed around the world, and are especially frequent in Spain, Australia and the USA. Disseminated infections are reported mainly in Spain and Australia, whereas in the USA most of the cases are localised osteoarticular infections, with a very small

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number of disseminated infections reported even in high risk groups (cancer and leukaemia patients) [14]. The present study was performed to assess if this irregular distribution could be a consequence of differing virulence of the strains. The main aim was to compare the virulence of strains from different geographical origins and clinical and environmental sources in a murine model. Two clinical strains of *S. apiospermum*, the other species of the genus also recognised as a common opportunist pathogen, were included in the study. This species is known to be less virulent, and could be more similar to the isolates of *S. prolificans* from the USA if they were demonstrated to be less virulent than the Spanish strains.

Materials and methods

Organisms

Ten *Scedosporium* spp. strains were used in the study (Table 1): eight isolates of *S. prolificans* (two from disseminated infections, two from localised infections, two from asymptomatic cystic fibrosis patients and two from the environment) and two isolates of *S. apiospermum* of clinical origin. All were isolated in Spain, except those from localised infections, which were provided by M. Rinaldi and were isolated in the southern USA. The strains were cultured on potato dextrose agar (PDA) for 7–10 days at 30°C. The inocula were prepared by flooding the surface of the agar plate with sterile saline, scraping the sporulating mycelium with a culture loop, and drawing up the resultant suspension with a sterile Pasteur pipette. The suspensions were then filtered once through sterile gauze to remove hyphae. The number of conidia in the suspensions was counted with a haemocytometer, adjusted to (8×10^5) – (1×10^6) conidia/ml and verified by plating dilutions of the suspensions on PDA plates.

Animals

OF1 male mice (Charles River, Criffa SA, Barcelona, Spain) with a mean weight of 31.5 g were used. Animals were housed eight per cage in standard boxes with corncob bedding and free access to food and water. Conditions were approved by the Animal Welfare Committee of the Faculty of Medicine of the university.

Infection

Inoculum suspensions (0.2 ml of 10^6 conidia/ml) of each fungal strain were injected intravenously *via* the lateral tail vein in groups of eight mice. Mortality was recorded daily for 30 days.

Histopathology

Representative portions of the organs were fixed in neutral buffered formaldehyde 10% for 10 days, embedded in paraffin wax and automatically processed. Sections (3 μm in thickness) of the embedded tissues were stained with methenamine silver (Grocott) for light microscopy observations.

Statistical analysis

Mean survival time (MST) was estimated by the Kaplan–Meier method and compared among groups by the log-rank test.

Results and discussion

The fungal inoculum used in this work was chosen in a previous experimental murine model which compared the mortality rate caused by several inocula of the strain FMR 3569 of *S. prolificans* ranging from 10^6 to 10^8 conidia/ml. The inoculum chosen was 10^6 conidia/ml, which provoked a 100% mortality in 2 weeks (data not shown).

The results confirmed the high virulence shown by *S. prolificans* in man, as most of the strains tested in the present study produced lethal infections in all the mice, with deaths occurring mainly from day 5 to day 15 (Fig. 1). Six of the eight strains of *S. prolificans* showed a very similar virulence pattern with no significant differences among them ($p = 0.1930$) (Table 2). These isolates were the two environmental strains from Spain (FMR 6721 with an MST of 12.00 days and 100%

Table 1. Isolates of *Scedosporium* spp. included in the study and their origins

Species	Strain	Origin	
S. prolificans	FMR 6721	Environment	Soil, Spain
	FMR 6802		Air, Spain
	FMR 6719	Pulmonary colonisation	Lung, Spain
	FMR 6642		Lung, Spain
	UTHSC 96-1714	Localised infection	Frontal sinus, USA
	UTHSC 98-1371		Maxillary sinus, USA
	FMR 6649	Disseminated infection	Blood, Spain
	FMR 3569		Blood, Spain
S. apiospermum	ACIA-F-303		Brain abscess, Spain
	FMR 4167		Otitis, Spain

FMR, Facultat de Medicina de Reus; UTHSC, University of Texas Health Science Center; ACIA, Asesoría Científica y de Investigación Aplicada.

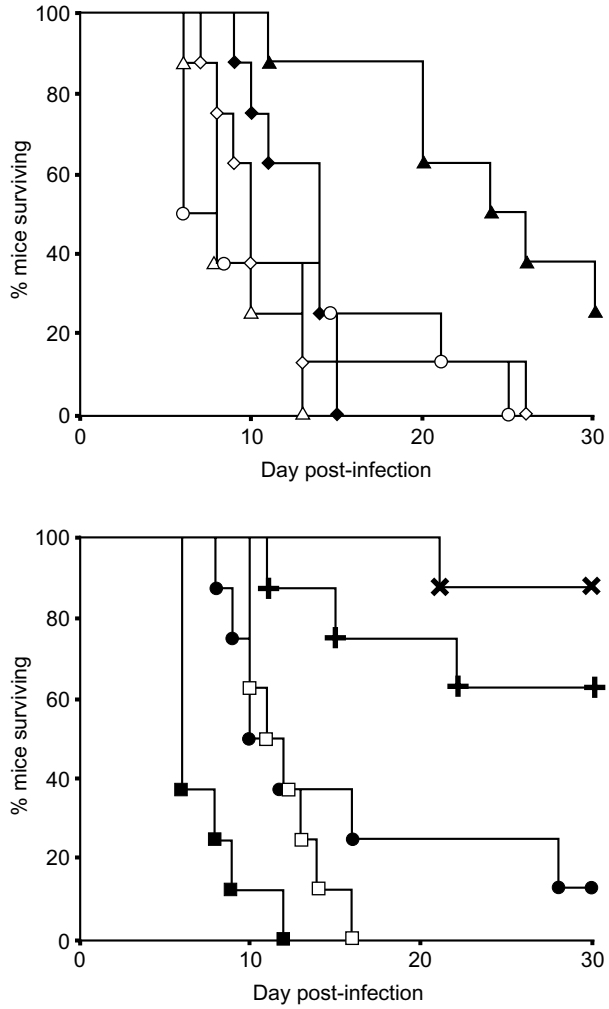

Fig. 1. Survival of mice infected with *Scedosporium* spp. Mice were infected with (1.6–2.0) $\times 10^5$ conidia and observed for 30 days. *S. prolificans*: environmental strains FMR 6721 (◇) and FMR 6802 (◆), strains from pulmonary colonisation FMR 6719 (△) and FMR 6642 (▲), strains from localised infections UTHSC 96-1714 (○) and UTHSC 98-1371 (●), strains from disseminated infections FMR 6649 (□) and FMR 3569 (■). *S. apiospermum* strains of clinical origin ACIA-F-303 (×) and FMR 4167 (+).

mortality, and FMR 6802 with an MST of 12.75 days and 100% mortality); the two American isolates (UTSCH 96-1714 with an MST of 11.50 days and 100% mortality, and UTHSC 98-1371 with an MST of 15.38 and 87.5% mortality); and one each of the colonising and disseminated infection isolates from Spain (FMR 6719 with an MST of 9.25 days and 100% mortality, and FMR 6649 with an MST of 12.00 days and 100% mortality), respectively. By contrast, two isolates clearly deviated from this group. They were strain FMR 6642 from pulmonary colonisation (MST of 23.88 days and 75% mortality), which was statistically less virulent than the main group referred to above ($p = 0.0009$), and strain FMR 3569 from disseminated infection (MST of 7.38 days and 100% mortality), which was statistically more virulent than the main group ($p = 0.0003$) (Table 2). According to these data, the strains of *S. prolificans* tested can be classified into three groups of virulence, containing those strains with high, intermediate or low virulence, respectively. The group with intermediate virulence contains six of the eight strains of *S. prolificans* tested, and the groups with higher and lower virulence contain only one strain each.

The two strains of *S. apiospermum* were clearly less virulent (MST of 26.81 days and mean mortality rate of 25%) than those of any of the three virulence groups of *S. prolificans* (MST of 7.38, 12.15 and 23.88 days and mean mortality rates of 100%, 97.9% and 75%, for the high, intermediate and low virulence groups, respectively) (Table 2). Similar results on the higher virulence of *S. prolificans* were obtained by Cano *et al*. [15], who compared the virulence of *S. prolificans* and *S. apiospermum* in a similar murine model. Drohuet *et al*. [16] tested a highly virulent strain of *S. prolificans* in murine and rabbit models and found close to 100% mortality with a low inoculum.

Although in this study, only a few strains have been compared because of the complexity of testing many animals, the results seem to demonstrate that Spanish isolates are no more virulent than those from the USA. Consequently, the reason why disseminated infections are more frequent in Spain than in the USA remains unexplained. It is possible that the clinical differences observed could be due to other factors, such as the different genetic susceptibility of people from different geographical regions. On the other hand, it seems clear that there are no differences in virulence between clinical and environmental strains and that any isolate, from the environment or from a clinical source, or

Table 2. *Scedosporium* spp. strains grouped according to their virulence in mice

Species	Degree of virulence	Strains	MST (95% CI) (days)	Mortality (%)	p value*
S. prolificans	High	FMR 3569	7.38 (5.85–8.90)	100	0.0003
	Intermediate	FMR 6721, FMR 6802, FMR 6719, UTHSC 96-1714, UTHSC 98-1371, FMR 6649	12.15 (10.57–13.72)†	97.9†	
	Low	FMR 6642	23.88 (19.13–28.62)	75	0.0009
S. apiospermum		ACIA-F-303, FMR 4167	26.81 (23.89–29.73)‡	25‡	0

MST, mean survival time in days (95% confidence interval).
*Significance *versus* the group with intermediate virulence in the log-rank test.
†Mean values for the six strains included in this group; strains were not statistically different among them ($p = 0.1930$).
‡Mean values for the two strains included in this group; strains were not statistically different among them ($p = 0.2511$).

from different geographical origins, can cause severe infections in a patient with predisposing factors. The presence of conidia of this fungus in the hospital environment, especially in those areas where neutropenic or severely ill patients are housed, can be extremely hazardous for those people. It is worth mentioning that the few existing reports on environmental isolates have been from potted plants in a hospital on two occasions [3, 17], and from air or dust samples in several Spanish hospitals that were undergoing building refurbishment [18, 19]. It seems important to include such species among the microorganisms that require special control measures in the atmosphere of critical areas of hospitals such as haematological wards, intensive care units or operating theatres. This is being done currently in most of the larger Spanish hospitals.

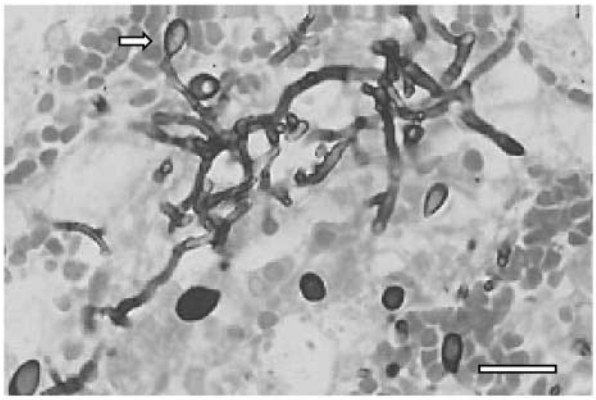

Fig. 2. Grocott-stained section of lung from a mouse infected with *S. prolificans*, showing hyphal elements and conidia (arrow). Bar = 10 μm.

One of the aspects of this study that, in our opinion, needs further work is the existence of a few strains of *S. prolificans* with significantly different virulence from that of the intermediate virulence group, which included most of the isolates. Only two of the eight strains tested had this significantly different virulence, but their atypical virulence could be precisely related to the disease they have provoked; the mildly virulent strain was isolated from a pulmonary colonisation, and the highly virulent strain was isolated from a fatal disseminated infection, both in Spain. This could indicate that strains with different virulence patterns can exist in the same region. However, further studies with a larger number of strains from both sources are required to ascertain this point. In a study on strains of *S. apiospermum*, differences in virulence were found between a strain from a patient with subcutaneous infection and one from the environment [20].

The fact that *S. prolificans* infections are relatively frequent in some geographical regions and very rare in others [5] is difficult to explain. Although only few data exist, the soil seems to be a natural reservoir for this species [5, 21], and it is probable that this fungus proliferates more under the conditions of a Mediterranean climate (hot dry summers and cool humid winters). This irregular distribution of the infections produced by *S. prolificans* is not found in those caused by *S. apiospermum*, the other species of this genus. The latter is also an important and relatively common opportunist fungus that can cause a wide spectrum of diseases in man, ranging from superficial infections in immunocompetent people to severely disseminated infections in immunosuppressed patients [22]. However, analysis of the published cases seems to indicate a more or less regular distribution of *S. apiospermum* throughout the world.

The fact that *S. prolificans* has been isolated only from air and soil could indicate that the wind could disperse the conidia from soil to the air and that the susceptible patient would be infected by inhalation of such propagules. This agrees with Gosbell *et al*. [3] who, on the basis of the frequent pulmonary involvement of the patients with *S. prolificans* infections, have argued that this could be the main portal of entry. The rapid dissemination of the fungus in the patients could be facilitated by its ability to produce adventitious sporulation, as happens with other fungi that cause hyalohyphomycosis, such as *Fusarium*, *Acremonium* or *Paecilomyces* spp. [23]. In the present study, it was common to find reproductive structures such as conidiogenous cells and conidia in the tissue of the infected mice that died (Fig. 2).

Another intriguing aspect of *S. prolificans* infections not yet explained is why these infections were not noticed until the beginning of the 1990s [24, 25]. It is probable that previous cases were confused with infections by *S. apiospermum* or even *A. fumigatus*. The clinical appearance of all of them is very similar and can be distinguished only by an accurate histological examination of the fungus in tissue (although this is not always successful), or by culture.

In conclusion, *S. prolificans* is a highly virulent opportunist fungus, as the clinical and experimental data demonstrate. Because of the resistance of this fungus to all available antifungal drugs, it is of enormous importance to develop preventive strategies focusing on reducing both environmental and host risk factors, including decreasing exposure of the airways and reducing the risk associated with neutropenia.

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