SHORT COMMUNICATIONS

Aspergillus fumigatus: A Possible Relationship between Spore Size and Virulence for Mice

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INTRODUCTION

Several workers have used the intravenous route of inoculation to study the dose response of mice to *Aspergillus fumigatus* spores. Scholer (1959) reported that six strains of *A. fumigatus* differed only slightly in virulence and Ford & Friedman (1967) found that three strains were comparable with each other in this respect. Smith (1972) concluded that four strains were of closely similar virulence but that a fifth strain which grew more slowly *in vitro* was less virulent; he also produced evidence suggesting that environmental factors significantly affected the susceptibility of mice. Ford & Friedman (1967) were unable to relate the virulence of 14 different *Aspergillus* spp. to spore characteristics such as germination time and size.

In preparing spore suspensions of five strains of *A. fumigatus* for inoculation, Smith (1972) found that optical opacity could be relied upon to indicate viable count sufficiently accurately for experimental purposes. The present communication reports the virulence of an unusual strain of *A. fumigatus* whose spores were large enough to produce suspensions which, when adjusted by optical opacity, possessed only about one-half the expected viable count.

METHODS

Organisms. The eight strains of *A. fumigatus* Fresenius on which the study was based were designated AF1 to AF8. Strains AF1 to AF5 (ATCC nos 28212, 28213, 28214, 28215 and 28216, respectively) have already been described by Smith (1972). Strains AF6, AF7 and AF8 were isolated from a blue grosbeak (*Guiraca caerulea*), an African sea eagle (*Haliaeetus vocifer*) and a parrot finch (*Erythrura psittacea*) respectively, each of which died with lesions of pulmonary aspergillosis on different occasions between 1971 and 1974 in the Zoological Society's collection at Regent's Park. All strains were purified and preserved at -20°C as described by Smith (1972); their identification as *A. fumigatus* was kindly confirmed by Dr R. R. Davies, St Mary's Hospital Medical School, London.

Measurement of spores. Observations were made on suspensions prepared as for inoculation into mice. Twenty spores of each strain were measured by means of a microscope with micrometer eyepiece.

Inoculation and examination of mice. The methods were those fully described by Smith (1972). Groups of female mice weighing about 20 g from an outbred closed colony were inoculated intravenously in dose volumes of 0.25 ml with nutrient-broth suspensions of spores of strains AF1, AF6, AF7 and AF8 obtained from 3 day cultures on Sabouraud's dextrose agar. The inocula were adjusted to contain appropriate numbers of viable spores; this was achieved by diluting suspensions standardized by Brown's opacity tubes (Wellcome Reagents) and the viable counts were checked retrospectively. The highest doses of the four strains contained comparable numbers of viable spores ranging from 6.7×10^6 to 10.2×10^6 , but because of the difference in spore size the associated optical opacity for strain AF8 was twice that for strains AF1, AF6 and AF7 (Table 2). Mice were examined by cultural methods after death or slaughter.

Table 1. *Spore sizes of A. fumigatus strains AF1 to AF8*

The dimensions given are based on measurement of 20 spores of each strain.

Strain	Spore length (μm)		Spore breadth (μm)	
	Range	Mean	Range	Mean
AF1	2.3-3.2	2.9	2.2-3.1	2.8
AF2	2.5-3.1	2.9	2.0-2.8	2.4
AF3	2.5-3.1	2.8	2.0-2.8	2.3
AF4	2.8-3.2	3.1	2.5-3.2	2.9
AF5	2.5-3.1	2.8	2.0-2.6	2.3
AF6	2.3-2.9	2.7	1.9-2.9	2.2
AF7	2.2-3.1	2.7	2.0-2.8	2.4
AF8	3.1-4.2	3.6	3.1-3.4	3.1

Table 2. *Virulence of strain AF8 and three other strains of A. fumigatus for mice*

Results show the numbers of mice that had died by 3 days and 28 days after infection, and, in parentheses, the numbers of survivors showing active *A. fumigatus* lesions at slaughter.

$10^{-6} \times$ dose of viable spores	3 days after infection with strain:				28 days after infection with strain:			
	AF1	AF6	AF7	AF8	AF1	AF6	AF7	AF8
6.7-10.2*	4/15	3/15	5/15	12/15	14/15(1)	12/15(1)	15/15	15/15
3.4-5.1	0/15	0/15	0/15	10/15	10/15(3)	12/15(2)	11/15(4)	15/15
1.7-2.5	0/15	0/15	0/15	3/15	8/15(3)	7/15(5)	7/15(5)	12/15(3)
0.8-1.2	0/10	0/10	0/10	0/15	5/10(5)	0/10(3)	4/10(5)	12/15(3)
0.4-0.6	0/10	0/10	0/10	0/10	2/10(3)	1/10(3)	1/10(3)	5/10(5)
0.2-0.3	0/10	0/10	0/10	0/10	0/10(2)	1/10(1)	3/10(1)	1/10(4)
0.1	NT	NT	NT	0/10	NT	NT	NT	1/10(3)

NT, Not tested.

* Inocula at the highest dose level had the opacity of Brown's tube 4 (strains AF1, AF6 and AF7) or tube 8 (strain AF8) and contained the following numbers of viable spores: AF1, 10.2×10^6 ; AF6, 6.7×10^6 ; AF7, 9.2×10^6 ; AF8, 8.2×10^6 .

RESULTS

Table 1 shows that the spores of strain AF8 were considerably larger than those of the other seven strains. Table 2 gives the results of the inoculation experiment. By 48 h after infection only one mouse had died; by 72 h, many of the mice inoculated with strain AF8 had died but few deaths had occurred among the mice inoculated with strains AF1, AF6 and AF7. The death rate had largely subsided 28 days after infection, at which time the superior virulence of strain AF8 remained clear. When the survivors were killed 28 days after inoculation, infection - if still present - was confined to the kidneys. Cultural examination of the survivors revealed that strain AF8 invariably produced infection when injected in doses of 0.5×10^6 or more spores, whereas the infectivity of strains AF1, AF6 and AF7 was much less uniform (Table 2).

In earlier studies (Smith, 1972) it was shown that strain AF1 was comparable in virulence with strains AF3, AF4 and AF5 and of greater virulence than strain AF2. It is therefore reasonable to conclude that strain AF8 was of greater virulence than the seven other strains AF1 to AF7.

DISCUSSION

Raper & Fennell (1965) gave the range of diameters for the spores of *A. fumigatus* as 2.5 to 3.0 μm , with extremes varying from 2.0 to 3.5 μm . The size of strain AF8 spores did not accord with these measurements and possibly the strain merits full description as a new variety.

The circumstances outlined suggest that the property of large spore size was in some way

associated with the unusually high virulence of strain AF8. However, the actual mechanism by which this high virulence was achieved is unknown. Further evidence is needed before a direct causal relationship between the easily observable characteristic of large spore size and the superior virulence of strain AF8 can be considered proved.

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