

An animal model of *Fusarium solani* endophthalmitis

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SUMMARY Infection with *Fusarium solani* was established by injecting spores into the anterior chambers of outbred Wistar rats. The disease produced was remarkably similar to that reported in man and progressed to a fulminating endophthalmitis. Attenuation of the disease could be accomplished by repeated animal passage of the isolate.

The increasing frequency of ocular fungal infections over the past decade has spurred interest in the development of more effective therapy. Most efforts have been devoted to clinical studies because of the lack of a suitable animal model of progressive ocular disease. Although observations of clinical cases have demonstrated the promise of certain antifungal agents, only in a reliable animal model of the disease can the problems of effective therapy be successfully elucidated.

Fusarium solani has recently emerged as an important ocular pathogen (Jones, D. B., *et al.*, 1969; Gugnani *et al.*, 1976), and a number of attempts have been made to establish an animal model. Transient disease has been produced in the rabbit but could not be maintained for more than a few days (Jones, B. R., *et al.*, 1969). Recently a more prolonged but still self-limiting disease has been reported in the rabbit pretreated extensively with corticosteroids (Forster and Rebell, 1975). Moreover Burda and Fisher (1959) reported the development of fusarial corneal infection in a corticosteroid treated rat. Despite the increased rate of infectivity in these models, a major criticism is the use of a compromised host.

The failure to develop a progressive eye infection in the normal animal emphasises the complexity of the problem. A number of factors involving the infecting organism and its interaction with the host animal appear to be important in this regard. In addition to variable pathogenicity of individual strains, maintenance of virulence in the laboratory is a major problem. Selection of an appropriate host animal is crucial. In this paper we report our experience with fungal endophthalmitis due to *F. solani* in the normal rat.

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Material and methods

SPORE SUSPENSION

Spores from *F. solani* isolates from human corneal ulcers were harvested by sterile loop from blood agar plates that had been incubated at 34°C for 3 to 4 days. The spores, suspended in sterile saline, were pipetted into a sterile tube and centrifuged for 10 minutes. After discarding the supernatant, fresh saline was added, and a second centrifugation was performed. The spore suspension was then brought to a total volume of 2 to 3 ml with sterile saline and counted by haemocytometer. Viable spore counts were determined by plating specific volumes of serial 1:10 dilutions in triplicate on to blood agar plates. These plates were incubated for 2 days at 34°C. Colony counts usually indicated at least 90% viability of the inoculum. Since *F. solani* cultures tend to lose their ability to sporulate with sequential passage, spores from the initial isolates were frozen for future use. Vials of these frozen spores were used periodically as required to ensure maximum sporulation. Fresh spore suspensions were made up to a concentration of 10⁸ or 10⁷/ml. Heated, killed spores and spores from isolates that had undergone animal passage 1 to 3 times were also used.

ANIMALS

Outbred Wistar rats of 200 to 300 g weight were used in our experiments and maintained for various intervals up to 3 weeks after inoculation.

INOCULATION TECHNIQUES

A 30-gauge needle was connected by plastic tubing to a 1 ml Hamilton gas type syringe, 1001-LT, fitted to a device which enabled us to dispense 0.02 ml of spore suspension per injection. The rats were anaesthetised with an intraperitoneal injection of 0.25 ml of pentobarbitone sodium. With the operating micro-

Table 1 Effects of inoculation with *F. solani*

Inoculum, spores	Number of animals	Culture positive, total cultures	Histology positive, total observed	Observations of inflammation		
				Acute/progressive	Transient	None
10 ⁸ /ml	78	33/38	27/30	61	10	7

scope for visualisation the 30-gauge needle was introduced into the anterior chamber through the peripheral cornea, and the spore suspension was injected. The spores could easily be seen entering the eye. Care was taken to ensure that the injection of the spores was performed as atraumatically as possible. If the tip of the needle touched the iris or lens or the injection was inadvertently delivered into the posterior chamber or the chamber became flattened after withdrawal of the needle, the animal was removed from the study. After each injection an aliquot was dispensed from the syringe for total spore counts and viability counts.

Biomicroscopic examination was performed on the rats 2 to 3 times a week, and the animals were killed at varying intervals over the ensuing 4 weeks. Eyes were submitted for culture and histopathological examination.

CULTURAL AND HISTOPATHOLOGICAL TECHNIQUES

We experimented unsuccessfully with several methods of obtaining material from each eye for simultaneous culture and histopathological examination. Therefore pairs of eyes matched for similarity of clinical disease were submitted for culture and histology. Eyes selected for culture were ground in sterile tissue grinder tubes, and the resulting fine suspension was cultured on blood agar, Sabouraud's agar supplemented with 100 µg/ml of gentamicin and 1% yeast extract, thioglycolate medium supplemented with gentamicin, and brain-heart infusion broth supplemented with gentamicin. All cultures were incubated at room temperature. Plates were observed for up to 2 weeks if necessary. Broth cultures were incubated on a rotary shaker.

HISTOLOGY

Eyes selected for histological examination were fixed in Bouin's fluid or 10% buffered formalin. Sections were stained with haematoxylin and eosin, methenamine silver, and periodic acid Schiff.

Results

EXPERIMENT 1

F. solani spores, in a concentration of 10⁸ spores/ml, were injected into the anterior chamber of one eye

in 78 rats. The eyes were observed for 3 weeks, the animals were killed at varying intervals, and the eyes were subjected to either fungal culture or histopathological examination.

CLINICAL OBSERVATIONS

Within 24 to 48 hours of inoculation a yellowish exudate developed on the iris adjacent to the pupil. Frequently associated with this was a posterior chamber hypopyon visible through the transparent iris. In some animals the exudate on the iris spread so that it partially or completely occluded the pupil. Corneal oedema developed as the chamber rapidly became shallower over the following few days. The eye then began to enlarge. However, in most animals with a posterior chamber hypopyon the inflammation remained posterior to the iris involving lens and vitreous. As the inflammation progressed, the anterior chamber became shallow and the eye enlarged. The time period for this course of events was rapid, usually from 3 to 10 days, but the process was usually well established by 7 days. In most of the animals the inflammation was so fulminating that a large proportion had to be killed prematurely. In 61 of the 78 animals this rather typical acute progressive anterior segment inflammation, with shallowing of the chamber, corneal oedema, and ocular enlargement developed. In 10 there was a mild to moderate inflammation of the anterior segment but the anterior chamber remained of normal depth. In 7 eyes there was no evidence of inflammation during the period of observation.

Overall, *F. solani* was isolated from 34 of 38 eyes cultured, while 27 of 30 matched eyes showed histological evidence of fungal replication based on the presence of hyphal elements in ocular tissue (Table 1). A disproportionate number of the animals were killed in the first week because of the severity of the inflammation. Table 2 summarises the results

Table 2 Isolate from human disease

Days after inoculation	4	7	14	21
Number of eyes	13	15	17	5
Positive cultures	6	7/9	12/14	1/2
Positive histology	7	3/6	2/3	3

Table 3 Effect of animal passage on clinical disease

Inoculum	Fresh isolate	Animal passage		
		X1	X2	X3
Number of eyes	15	8	4	4
Severe progressive inflammation	14	2	1	0
Culture positive	6/6	1/1	0/1	—
Histology positive	6/7 (Day 4–15)	0/1 (Day 8)	— (Day 4)	—
Mild to moderate inflammation	1	6	3	4
Culture positive	0/1	3/3	2/2	2/4
Histology positive	— (Day 21)	2/3 (Day 8–21)	1/1 (Day 7–11)	— (Day 14)

in animals killed at days 4, 7, 14, and 21 only. The fungus was recovered from 12 of 14 eyes cultured at 2 weeks.

EXPERIMENT 2

In an attempt to reduce the severity of the inflammation the concentration of inoculum was reduced to 10^7 /ml. The experimental technique was otherwise identical. In contrast to the previous experiment we were unable to produce disease in 21 of the 31 inoculated eyes, and in the remainder 2 were culture positive and 4 were histologically positive for fungal infection.

EXPERIMENT 3

Since animal passage may reduce the virulence of the organism and therefore the severity of the ocular disease, spores harvested from an isolate from human corneal disease were passaged 3 times in rat eyes. Inocula were prepared for injection from isolates after each passage. Thus, we compared the disease produced by the fresh isolates with that produced by the same isolate after 1, 2, and 3 animal passages. The results are shown in Table 3. The original human isolate produced severe progressive inflammation, so that it was necessary to kill all but one of the animals in the first 2 weeks. Animal passage caused marked attenuation in the disease process, so that it could now be classified as mild to moderate. Nonetheless, recovery of the isolate by culture or histological confirmation of a fungal infection continued to occur in a high percentage of the animals (Table 3). We were unable to show that repeated animal passage had a greater attenuating effect than a single passage.

EXPERIMENT 4

The injection of heat inactivated spores into 11 eyes

produced a transient severe inflammation in 3 eyes and minimal inflammation in 2, and the remaining 6 eyes were unaffected. All cultures were negative for fungi.

HISTOLOGICAL CONFIRMATION OF FUNGAL INFECTION

Histological examination revealed the presence of hyphal fragments in a variety of ocular structures in 27 of 30 eyes. The fungus showed an overwhelming tendency to localise in the posterior chamber of the rat eye and lens, but was also found invading the vitreous, iris, ciliary body, anterior chamber, and cornea. The histological features will be discussed in more detail in a forthcoming paper (in preparation).

Discussion

An animal model of human infectious disease must meet certain criteria. It should simulate the human disorder and be precise and reproducible. The disease should be progressive rather than self-limited and should be accessible to clinical and laboratory investigation. Finally, modification of the host, for example, by immunosuppression, should be avoided if at all possible.

The rat model of infection with *Fusarium solani* appears to meet these criteria. In these experiments the animals developed an endophthalmitis that was remarkably similar to the human disease, although somewhat more severe (Gugnani *et al.*, 1976). Nonetheless, it was sufficiently even-paced to permit sequential clinical observations to be made. Animal passage of the isolate performed to decrease its virulence was successful in attenuating the clinical disease, but reduction in the concentration of the inoculum resulted in failure to establish an ongoing infection in most animals.

Although the rat eye is small, it is easily examined by slit lamp. The transparent iris permits some assessment of the inflammatory changes in the posterior chamber, including the development of hypopyon—a unique observation, we believe. Studies are now under way to evaluate the various forms of therapy of infection with *F. solani* through the use of this model.

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