Vaccination procedures and the infectivity of dermatophyte lesions

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Abstract

By means of an experimental guinea pig model, the immunogenicities of a live spore vaccine, a killed hyphal cell wall vaccine and a soluble cytoplasmic extract vaccine of Trichophyton mentagrophytes var. erinacei were compared, and their modifying influence on the infectivity and duration of experimental dermatophyte lesions examined.

Of the three vaccines, the live-spore one was the most effective and closely simulated the type of immunity which develops following a natural infection. Lesions produced in cell wall vaccinated animals revealed significantly less infectivity and were reduced in duration as compared with lesions in unvaccinated animals. The cytoplasmic extract vaccine had no beneficial effect on the course of experimental infection; indeed it seemed to increase the infectivity of lesions.

Acquired resistance to reinfection in a pregnant guinea pig was not passed on to the offspring.

Significant levels (titer of ≥1:16) of antibody were detected in the sera of all infected and vaccinated animals using an indirect fluorescent antibody technique and germling antigens. Using counterimmunoelectrophoresis, precipitating antibodies were detectable in the sera of all seven animals vaccinated with the cell wall or cytoplasmic extract, in one (20%) of the control animals after reinfection, and none of the four animals injected subcutaneously with the live spore vaccine. Thus no correlation between antibody titer and the severity and duration of lesions was observed.

These results endorse the growing hypothesis that cell mediated immunity is of prime importance in protection against dermatophyte invasion and suggests that prophylactic vaccination procedures are worthy of further evaluation in lower animals.

Introduction

In many areas of the world, ringworm in lower animals constitutes a serious public health problem. Its toll in terms of suffering, disability, man hour losses, psychological trauma and monetary expenditure is much greater than is generally realised. Effects on the animals themselves may also be dramatic with a lowering of the value of pelts and hides, and a loss in condition of infected animals (18). Even though our awareness of this problem has been heightened in recent years, the total effort given to the control of lower animal ringworm has been minimal.

Since the beginning of the present century, sporadic experimental and clinical investigations have been carried out on the immunological aspects of dermatophyte infection – often with conflicting results (5, 13). Early investigators in the immunization field were able to demonstrate resistance to reinfection following recovery from a primary infection; they believed that this immunity could be induced consistently only following active cutaneous disease. Subsequently it has been demonstrated...
that a state of relative immunity can be induced by techniques other than active infection. These include repeated intradermal or subcutaneous injections of fungal extracts and/or suspensions; subcutaneous inoculation of dead or viable spores; and repeated topical application of a macerated fungal suspension in an ointment base. Of these techniques, the use of a live vaccine prepared from a readily sporing strain of *Trichophyton verrucosum* (as *T. faviforme*, TF-130) by Sarkisov and his colleagues, has apparently been highly successful in lowering the incidence of bovine ringworm in the USSR (17). A lyophilized version of this vaccine (LTF-130) is now being used in numerous areas of eastern Europe and the Soviet Union (7, 9, 10, 17, 23).

In 1979, Chittasobhon and Smith (3) described a guinea pig model in which the infectivity of experimental dermatophyte lesions could be assessed quantitatively using a hair brushing technique. The following paper describes the application of this technique to the investigation of experimental ringworm lesions in guinea pigs following a series of attempted vaccination procedures.

**Materials and methods**

**Animals**

Guinea pigs, aged between 5 and 10 weeks, were used in all experiments. They were housed in separate cages and fed water and pellets as required.

**Fungus**

The dermatophyte used in all experiments was *Trichophyton mentagrophytes* var. *erinae* (TE). This strain had originally been recovered from a hedgehog in New Zealand and had been maintained by regular subculturing on Sabouraud's dextrose agar plates.

**Preparation of spore suspension/inoculation of guinea pigs**

These were carried out as previously described (3). Primary infection was confined to the right flank of each animal; reinfection was attempted on the opposite (left) flank.

**Vaccination procedures**

Twelve guinea pigs were divided into 3 groups, each group being "vaccinated" with a different antigen – namely live spores (5 animals), a formalinised cell wall suspension (3 animals), and a soluble cytoplasmic extract (4 animals).

1. **Preparation of cell wall suspension.** Approximately $10^6$ TE spores were inoculated into 500 ml of Sabouraud's dextrose broth containing 0.05 mg/ml chloramphenicol. After incubation in a shaking water bath at 30 °C for 4 days, the mycelial mat was removed by filtration through sterile gauze. After washing with 500 ml of physiological saline, the hyphae were frozen at −80 °C overnight. They were then thawed and ground in a sterile mortar for 20 minutes. The freezing/grinding process was repeated another two times. The resulting suspension was then centrifuged at 10,000 rpm for 20 minutes to separate the cell wall from the cytoplasm extract. To further free the cell walls of cytoplasmic material, the cell wall pellets were resuspended in 0.01 M potassium phosphate buffer (pH 7.2), containing 100 μg of trypsin (Difco) per ml, to approximately 10 times their packed volume. The suspension was incubated for 3 hours in a shaking water bath at 30 °C. The trypsin-treated cell walls were then washed in sterile distilled water and centrifuged at 10,000 rpm for 20 minutes. This washing procedure was repeated 3 times. One percent formalin (final concentration) was then added to the cell wall pellets which were then left at 4 °C for 24 hours. These were then washed 3 times with sterile distilled water. Culture techniques revealed the suspension to be nonviable. The formalinised cell wall suspension was finally lyophilized and kept at room temperature.

For vaccination purposes, the formalinised cell wall suspension which had been reconstituted in saline to give a final concentration of 5 mg/ml, was injected subcutaneously (1.0 ml volume) into the flank of each of the 3 guinea pigs. A week after the primary inoculation, a booster dose of another 5 mg/ml antigen was given to each animal. This was followed by a final booster of the same concentration of antigen 2 weeks later.

2. **Preparation of cytoplasmic extract.** Flasks of broth were inoculated with spores and incubated as