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Brief Communication

Establishment of *Pneumocystis carinii* Infection in a Rat Population

Pneumocystis carinii (PC) is an opportunistic pathogen with uncertain taxonomy, which frequently causes clinical diseases in immunocompromised hosts. The organism is difficult to cultivate in vitro. To study the basic biology of PC the method of choice has been to induce acute PC-pneumonia, by what is believed to be a reactivation of a latent infection in animal models, using corticosteroid immunosuppression. Rats and mice have been the most commonly used experimental animals. Normally, a manifest disease in rats is produced in 6–8 weeks of immunosuppression.

In recent years difficulties in induction of a manifest PC infection by immunosuppression have been reported (*Bartlett* 1987, *Eisen* 1989). The infective agent is airborne, but the infective form of the organism has not been identified (*Hughes* 1982, *Walzer & Cushion* 1989). Unsuccessful manifestation of PC-infection is believed to be due to the fact that test animals have been free of the latent infection, or that the unidentified infective form of the organism has not been present in the laboratory environment.

We first attempted to manifest an infection in our own laboratory rat colony. When it became obvious that the animals did not produce a manifest infection, we decided to study whether the infection was transmissible to our rat colony. The second experiment is referred to here as the transmission experiment.

In the first experiment 12 Wistar rats, 8 male and 4 female, were used. In the transmission experiment the recipient group consisted of 9 rats, 4 male and 5 female, these Wistar rats being part of a stock bought from one supplier (National Laboratory Animal Center, Kuopio, Finland) 2 years earlier and since then bred without any contact with other animals. Two Sprague-Dawley rats from a colony known to be PC-infected (University of Costa Rica, San Jose, Costa Rica) acted as sources of the infection.

An immunosuppression regimen using methylprednisolone (Depo-Medrol®) 16 mg/kg, injected subcutaneously once a week was used for all animals. In the first experiment we fed them a low protein diet (6 %) without any antibiotic prophylaxis, but because severe deterioration of physical condition and deaths due to *E. coli* nephritis occurred in these animals after 36 days (Fig. 1), the regimen was changed in the transmission experiment to a normal diet with tetracycline (500 mg/l) in drinking water for controlling secondary bacterial infections. Animals were kept in ordinary cages; in the transmission experiment carrier animals were kept in the same room as the recipient animals, cages close to each other. Infected rats were in air contact with the recipient animals for 39 days.

The caudal right pulmonary lobe was used for the preparation of imprint slides and for preparing frozen sections. The rest of the lungs were frozen and kept at –20°C for quantification procedures. Lung imprints were stained with toluidine blue O, and frozen sections stained using a modification of the Grocotte silver methenamine method. In the quantification procedure approximately

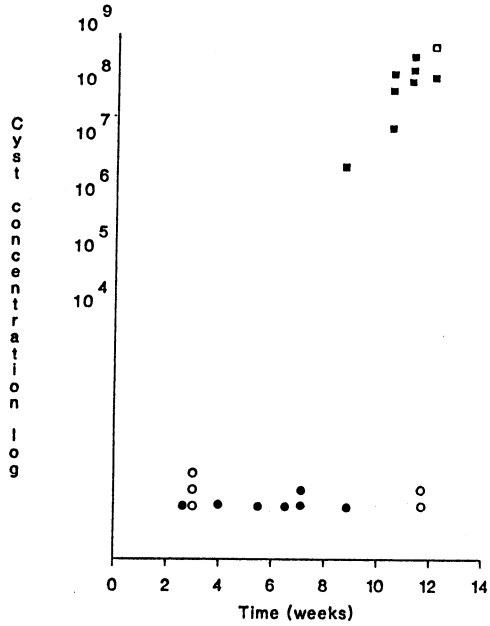

Figure 1. Survival time (in weeks) and cyst-concentration (cyst/g of lung tissue). All animals received methylprednisolone parenterally (16 mg/kg), Wistar rats in transmission experiment (■, □), in original experiment (●, ○). Spontaneously died animals (■, ●) and sacrificed (□, ○).

0.1 g samples of lung tissue were cut into small pieces and digested in a tenfold volume of 0.1 % collagenase at 40°C for 45 min with magnetic stirring. After centrifugation at 3000 g for 20 min, the pellet was washed with 0.15 M NaCl, centrifuged again, and diluted 1:10, 1:100, and 1:1000. A 10 µl sample of each dilution was air dried on a microscope slide to form a "spot" which was fixed in absolute ethanol for 10 min and stained with toluidine blue O. The number of cysts per microscopic field along the diameter of the sample spot was registered and the concentration of cysts calculated.

Only the animals in air contact with the imported rats from Costa Rica developed *Pneumocystis carinii* pneumonia (PCP). Cyst concentration was higher in those recipient animals which survived longer (Fig. 1) and highest in carrier animals ($7.5*10^8$ cyst/g lung tissue and $8.4*10^8$ cyst/g, respectively).

Mean survival time of animals in the first experiment was 43 days and in the transmission experiment 78 days (excluding euthanized animals). All samples with detectable PC in toluidine blue staining were also positive with methenamine silver.

The need to control secondary infections in immunosuppressed animals became obvious from the fact that only 3 animals in the first experiment survived as long as the first fatal cases of PCP in the transmission experiment. Thus we believe that the follow-up time in the first experiment was sufficient to show that the rat colony was previously free of the infection and that the manifest infection seen in the transmission experiment was dependent on the availability of the organism from the surroundings.

Weight-loss was very obvious during the whole experiment in both groups. The animals which died spontaneously in the first experiment had lost 40.9 % of their weight, and in the contact group the weight loss was 39.9 %. This was mainly due to the catabolic effect of methylprednisolone and the low-protein diet in the first experiment.

Our results support the theory of the airborne route of infection. We also show that animals initially free of a latent infection were susceptible to the infection. In conclusion this means that the infective form of the organism was not initially present in the laboratory environment, and rats were not genetically resistant to PC infection. If manifest PCP can not be induced by immunosuppression of a original rat colony, a

simple way to establish the animal model is to house immunosuppressed rats in close contact with rats known to be PC infected.

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References

Bartlett MS, Durkin MM, Jay MA, Queener SF, Smith JW: Sources of Rats Free of Latent Pneumocystis carinii. J. Clin. Microbiol. 1987, *25*, 1794–1795.

Eisen S: Decreased Yield of Pneumocystis carinii from Cortisonized Rats. J. Parasitol. 1989, *75*, 82–85.

Hughes WT: Natural Mode of Acquitition for de Novo Infection with Pneumocystis carinii. J. infect. Dis. 1982, *145*, 842–848.

Walzer PD, Cushion MT: Immunobiology of Pneumocystis carinii. Pathol. Immunopathol. Res. 1989, *8*, 127–140.

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