Exposure of Guinea Pigs to *Rickettsia rickettsii* by Aerosol, Nasal, Conjunctival, Gastric, and Subcutaneous Routes and Protection Afforded by an Experimental Vaccine

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Received for publication 28 March 1979

Guinea pigs were inoculated with Rocky Mountain spotted fever by the aerosol, conjunctival, subcutaneous, intragastric, and intranasal routes. Rickettsial infection was produced by all routes except intragastric. All animals with clinical signs of disease developed agglutinating antibody, and most developed a cell-mediated immune response. Disease produced by all experimental routes (except intragastric) was indistinguishable. The tissue culture-derived inactivated vaccine produced in this laboratory protected guinea pigs against an aerosol challenge.

Although Rocky Mountain spotted fever (RMSF) infection is naturally transmitted by the bite of a tick (intradermal), there is increasing evidence that aerosols may be the primary means of transmission among laboratory workers. Oster et al. (11) reported on nine cases among laboratory workers with no known intradermal exposure, all of whom were potentially exposed to aerosols. Additionally, the question of possible infection of workers by other routes such as ingestion or intraocular instillation is occasionally encountered.

RMSF can be induced experimentally in guinea pigs by subcutaneous or intraperitoneal inoculation; the clinical features and pathological lesions are similar, if not identical to those seen in humans (9, 16). Saslaw and Carlisle reported experimental aerosol infection of rhesus monkeys with *Rickettsia rickettsii* (13); again, clinical features and pathological lesions were nearly identical to those seen in intraperitoneally infected monkeys (10) and in naturally occurring disease in humans (17). Similar aerosol studies have not been done in guinea pigs. The purpose of this investigation was to characterize aerosol infection of guinea pigs with *R. rickettsii* and to determine whether infection could be induced by intranasal or conjunctival inoculation or by ingestion of viable organisms.

Presently, there are no commercially available RMSF vaccines. A yolk sac-grown, Formalin-inactivated vaccine previously used in adults and children at risk was found to be inefficacious in human challenge trials (2). In our laboratory, a chicken embryo cell-grown, Formalin-inactivated RMSF vaccine suitable for human immunization has been prepared (6); its efficacy has been tested in rhesus monkeys (7, 12), and its safety and immunogenicity has been tested in humans (1). Another purpose of the experiments presented here was to test the efficacy of this vaccine in guinea pigs against aerosol challenge with RMSF.

MATERIALS AND METHODS

*Rickettsiae*. Master seed of Sheila Smith strain of *R. rickettsii* was propagated in embryonated chicken eggs (14); working seed was grown in duck embryo cell cultures (4). Rickettsiae were titrated by the plaque test described by Weinberg et al. (15).

Test animals. Outbred male Hartley strain guinea pigs, weighing 350 to 450 g, were obtained from Buckberg Lab Animals, Tompkins Cove, N.Y. All animals were provided water and commercial guinea pig chow ad libitum.

Test plan. Guinea pigs (four per group) were exposed to *R. rickettsii* by the following procedures. (i) In the aerosol procedure, animals were exposed for 16 min to aerosols of rickettsiae disseminated by a Collison atomizer (8); they were then housed in safety cabinets. Two groups of four animals each were exposed to noninfected duck embryo cell-grown material by the same method to serve as sham controls. Aerosol samples, collected in AGI-30 samplers containing Earle medium 199 supplemented with 5% fetal calf serum were stored at −70°C until assayed for viable rickettsiae. (ii) In the intranasal procedure, guinea pigs were anesthetized with halothane and then challenged with 100 μl of selected dilutions of rickettsial suspensions. (iii) In the conjunctival procedure, 50 μl of a rickettsial suspension was dropped onto the conjunctiva of guinea pigs. (iv) In the intragastric procedure, guinea pigs were anesthetized with halothane; the inoculum was introduced into the stomach through a 1-mm flexible tube. (v) In the subcutaneous procedure, for comparative purposes, guinea pigs were inoculated with 0.5 ml of appropriate dilutions of rickettsiae.
RESULTS

Clinical responses of guinea pigs infected by the various routes and doses are presented in Table 1. Immune responses are shown in Table 2. None of the guinea pigs exposed to sham material became clinically ill or developed humoral or cell-mediated immune responses. The severity of illness in guinea pigs which became clinically ill was the same regardless of route or dose of inoculum.

Development of illness depended upon an interaction of route of inoculation and dosage. No guinea pigs challenged intragastrically became ill. In contrast, all animals given 10^6 organisms in the conjunctiva or intranasally became ill, but none of the four animals inoculated conjunctivally and only one of four instilled intranasally became ill after challenge with 10^6 rickettsiae. All animals given as few as 80 rickettsiae by aerosol became ill, and even one of four given a calculated dose of less than one rickettsia had signs of disease. All guinea pigs vaccinated with our chicken embryo culture-grown vaccine were protected when challenged by aerosol or subcutaneous route.

Guinea pigs with signs of disease consistently developed microagglutination antibody, and most also demonstrated a significant lymphocyte transformation response. Guinea pigs that did not show signs of illness were susceptible to infection when rechallenged; therefore, there were no inapparent infections that stimulated immunity. None of the guinea pigs which had signs of RMSF and recovered was susceptible to subsequent intraperitoneal rechallenge with 10^6 plague-forming units of *R. rickettsii*.

Seven guinea pigs exposed to an aerosol dose of 10^6 rickettsiae were killed at selected intervals, necropsied, and examined histologically. Vascular lesions typical of RMSF in guinea pigs (9) were observed, primarily in the cremaster muscle and testis on days 8 and 15 and to a lesser extent.
extent in the dermis of all skin sections. Vascular lesions were characterized by segmental infiltration of the vessel wall by neutrophils and mononuclear cells, fibrinoid necrosis, endothelial proliferation, and perivascular infiltration by macrophages and lymphocytes. No pulmonary lesions attributable to rickettsial infection were observed.

DISCUSSION

Since accidental laboratory infections appear to result frequently from aerosol generation, availability of a reliable animal model for such exposures is of increasing concern. Due to escalating costs and decreasing availability of rhesus monkeys, there is increasing dependency on guinea pigs for studies with RMSF.

From the present studies, it appears that guinea pigs can be consistently infected with *R. rickettsii* presented as an aerosol and the resulting disease produced is indistinguishable from that produced by other routes. The fact that infection can occur by intranasal and conjunctival routes is mainly of academic interest, but could be an important consideration as possible laboratory exposures. However, since animals were exposed to whole-body aerosols, it is possible that such a procedure led to infection by intranasal, conjunctival, or even intragastric routes. The guinea pig aerosol model has value as an alternate mode of infection in examining spotted fever disease processes and methods of altering them. Additionally, we have also demonstrated that the chicken embryo culture-grown vaccine protects guinea pigs against an aerosol challenge. This is significant since laboratory workers potentially exposed by aerosol generation will most likely be the first at-risk group immunized with the vaccine.

LITERATURE CITED


