Effect of Vaccination on Feline *Chlamydia psittaci* Infection

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Experimental ocular infection of specific-pathogen-free cats with the feline pneumonitis strain of *Chlamydia psittaci* produced an acute, severe conjunctivitis characterized by blepharospasm, conjunctival hyperemia, chemosis, and ocular discharge. Organisms were recovered from the conjunctiva for several weeks, and persistent genital and gastrointestinal infection also resulted from the ocular infection in some cats. Subcutaneous vaccination with live feline pneumonitis *C. psittaci* 4 weeks before ocular challenge significantly reduced the severity of the conjunctivitis. However, there was no effect on shedding of organisms from the eye or on the transmission of infection to the gastrointestinal and genitai tracts. It is suggested that the acute stage of this ocular disease is caused largely by release of pathogenic antigen(s) from chlamydia-infected conjunctival cells, rather than by a direct cytopathic effect of chlamydial replication. Thus, vaccination with whole live organisms reduced the acute disease in experimentally infected cats but did not prevent shedding of the organism. The implications of these findings are discussed.

*Chlamydia psittaci* was isolated and identified from the pneumonic lung of a naturally infected cat in the United States in 1942 (1). It was the first pathogen to be isolated from cats with respiratory disease, and the disease syndrome was designated feline pneumonitis (FPn). The disease was characterized by sneezing and coughing, accompanied by mucopurulent ocular and nasal discharges (2). Subsequent work has shown that feline *C. psittaci* is primarily a conjunctival rather than a pulmonary pathogen and that it produces chronic conjunctivitis (9). The organism has been isolated from cases of feline conjunctivitis in the United States (3), Canada (20), Australia (24), and the United Kingdom (5, 10, 27), and it has been recovered from the genital tract as well as from the eye (5). This organism therefore bears resemblance to other chlamydial strains which infect the mucosal surfaces of the eye and genital tract, such as the guinea pig inclusion conjunctivitis strain of *C. psittaci* (16) and ocugential serotypes of *Chlamydia trachomatis* (25).

Previous reports on the effect of vaccination on this infection have produced conflicting results (4, 13, 14, 21). In one study, cats were vaccinated intramuscularly with a modified live chicken embryo-origin commercial vaccine and were aerosol challenged 30 days later with yolk sac-grown FPn. The vaccine modified the disease in vaccinated cats in that rectal temperatures were lower and clinical signs of serous rhinitis and conjunctivitis were more prevalent in unvaccinated controls. However, the organism was isolated from some vaccinated cats 27 days after challenge, and complement fixation titers had no apparent correlation with protection (14). In another study, several different preparations of vaccines were tried, which contained either inactivated or live organisms, propagated in yolk sacs, allantoic fluid, or mouse lungs (13). The vaccine was given subcutaneously, and challenge was by intranasal administration of infectious yolk sac material given 5 weeks later. The vaccines which contained live organisms gave a greater degree of protection than those which were inactivated, and the majority of vaccinated kittens were clinically protected. Other workers have reported partial protection (21), whereas in another study no protection could be demonstrated (4).

The aim of this work was to evaluate a live *C. psittaci* vaccine, commercially available in the United States, for protection of cats against experimental ocular challenge with a recent British isolate of FPn.

**MATERIALS AND METHODS**

**Culture of *C. psittaci***. Isolation of chlamydiae was attempted in cycloheximide-treated McCoy cell monolayers prepared on 10-mm sterile glass cover slips in flat-bottomed plastic tubes. McCoy cells were maintained as described previously (18). Culture tubes were seeded with 2 × 10⁵ cells in 1 ml of growth medium and incubated at 35°C for 24 h. Each cell culture was inoculated with 0.25 ml of material. The tubes were centrifuged at 3,000 × g for 1 h at 30°C and then incubated at 35°C for 1 h. The medium in each tube was then replaced with 1 ml of maintenance medium which contained 1 µg of cycloheximide. The cultures were incubated at 35°C until 44 h post centrifugation. The supernatant was then removed, and the monolayers were washed gently once with phosphate-buffered saline, pH 7.2, and then fixed in methanol for at least 10 min. Monolayers were stained by indirect immunofluorescence with serum from a rabbit immunized against FPn and with fluorescein-conjugated antirabbit immunoglobulin serum (Wellcome). The cover slips were mounted, cell side downwards, on glass slides (0.8 to 1.00 mm thick) in phosphate-buffered saline–glycerol (1:1). Each monolayer was scanned by fluorescence microscopy at 200× magnification. The FPn strain produces lobulated or multiple inclusions in cycloheximide-treated McCoy cells (22); therefore, infected cells rather than inclusions were counted, and infectivity was expressed as infected cell-forming units (ICU) per milliliter. When no more than 100 cells of the monolayer were infected, the whole cover slip was scanned and the infected cells were counted. Above 100 infected cells, a mean of 10 fields (selected from different...
areas of the monolayer) were counted, and the total cover slip count was calculated by extrapolation.

**Vaccine.** The vaccine was a commercial live vaccine, modified from chicken embryo origin (Psittacid; Fromm Laboratories Inc., Grafton, Wis.) The *C. psittaci* strain used in this vaccine was the Baker strain (1), which was provided in freeze-dried vials. One milliliter of reconstituted vaccine had a titer of \(5 \times 10^4\) ICU/ml in cycloheximide-treated McCoy cells. Each dose of vaccine was reconstituted immediately before use in the diluent provided.

**Challenge isolate.** The FPn-Pring 1 strain of *C. psittaci* used for challenge was isolated in 1981 from the conjunctiva of a 7-week-old Siamese kitten with conjunctivitis. The kitten came from a colony of cats in Somerset, England. Primary isolation was in emetine-treated McCoy cells (27), and this was confirmed by reisolation in the yolk sacs of embryonated chicken eggs. A tissue culture pool of FPn-Pring 1 was used as the challenge inoculum, which was diluted to \(10^6\) ICU per 25 μl in phosphate-buffered saline before use.

**Experimental procedure.** Ten 12-week-old specific-pathogen-free cats were used. They were housed in two groups, and each group consisted of four female and one male cat. On day 0, all cats in one group received 1 ml of the vaccine by subcutaneous injection. To monitor possible excretion of the vaccine chlamydiae, conjunctival and rectal swabs were taken from all five cats, and vaginal swabs from the four females, on days 2, 6, 8, 9, 12, 14, 16, and 29 postvaccination. Cats in both groups were given a 25-μl drop of challenge chlamydiae conjunctivally 29 days after vaccination.

**Clinical observations.** Clinical signs were scored daily for the first 30 days postchallenge (pc) and then twice weekly up to 80 days pc. Relevant clinical features, which included chemosis, conjunctival hyperemia, and serous and mucopurulent ocular discharges, were each scored on a seven-point scale, from 0 to 3. Each eye was scored separately.

**Sample collection.** Conjunctival, vaginal, and rectal sample swabs for FPn isolation were placed in 2 ml of chlamydia transport medium (23) and stored at −70°C before inoculation onto cycloheximide-treated McCoy cells. Swabs were taken every 2 or 3 days until 30 days pc and then once weekly until 89 days pc and intermittently thereafter until 8 months pc. Conjunctival swabs were taken from the right eye only in order to assess the effect of swabbing on the clinical eye score. FPn excretion from the right eye of each cat was assessed by estimating the ICU per 0.25 ml of clinical specimen. Cats were also monitored by oropharyngeal swabs for feline respiratory viruses (17) and by conjunctival swabs for bacterial pathogens by conventional techniques.

**Serological studies.** Sera were collected prevaccination (where relevant), prechallenge, and 1, 2, 4, 8, 17, and 37 weeks pc. Sera were tested for chlamydial antibodies with a homologous indirect immunofluorescence (IF) technique, in which whole inclusions of FPn grown in cycloheximide-treated McCoy cells on Teflon-coated slides were used as the antigen. In addition, the sera were also screened for complement-fixing (CF) antibodies with a 4-volume (0.025 ml/volume) micromethod and overnight fixation at 4°C.

**Statistical methods.** Clinical scores obtained from the right and left eye of each cat were compared by the Wilcoxon matched-pairs signed-ranks test (26). Combined clinical eye scores for right and left eyes were used in comparisons between vaccinated and unvaccinated groups. Statistical analysis of clinical scores for conjunctival hyperemia and ocular discharge between the two groups was carried out by the Mann-Whitney U test (12).

**RESULTS**

**Vaccinated cats prechallenge.** All cats appeared healthy prior to challenge, and chlamydiae were not isolated from any conjunctival, vaginal, or rectal swabs taken after vaccination.

**Clinical disease pc.** Total clinical eye scores for each cat are shown in Fig. 1, and median scores for conjunctival hyperemia and total ocular discharge in the two groups are summarized in Fig. 2. Unvaccinated cats (Fig. 1, a to e) developed severe conjunctivitis with blepharospasm, conjunctival hyperemia, chemosis, and serous and mucopurulent ocular discharge 4 days pc. In addition, some cats also developed mild respiratory signs which included slight nasal discharge, occasional sneezing, and submandibular lymph node enlargement. Marked conjunctival hyperemia persisted for 30 days pc and then gradually subsided. Ocular discharge reached a peak at day 11 pc and then decreased.

In the vaccinated cats (Fig. 1, f to j), illness started 1 day later and was much less severe. The clinical picture consisted only of mild to moderate conjunctivitis, with slight mucopurulent ocular discharge and no serous ocular discharge. There was no blepharospasm, chemosis, nasal discharge, sneezing, or lymph node enlargement.

Combined eye scores for right and left eyes were used in comparisons between vaccinated and unvaccinated groups. Despite the small number of animals in each group, there was a significant difference in conjunctival hyperemia between the two groups on days 4 to 30 pc \((P = 0.05)\) and a significant difference in ocular discharge on days 4 to 16 pc \((P = 0.05)\).

When the clinical scores for the right and left eyes of each cat were compared, there was no significant difference between each eye in two of five vaccinated cats and four of five unvaccinated cats. In the remaining four cats the clinical scores for the right eyes were significantly worse than for the corresponding left eyes \((P < 0.05)\). Thus, trauma associated with swabbing the right eye may have increased the clinical disease in some but not all cases.

**Isolation of chlamydiae.** Excretion from the conjunctiva was first detected 2 to 4 days pc. Despite reduction of clinical disease in vaccinated cats, the number of organisms shed from the eye in the acute stage of illness was not reduced. Conjunctival excretion was more prolonged in the vaccinated cats (63 days pc for three cats, 68 days for two cats) than in the unvaccinated group (29, 35 [two cats], 42, and 46 days pc) for the period of the study (Fig. 1 and 2). However, organisms were subsequently recovered intermittently from some cats in both groups between 3 and 8 months pc (J. Wills, Ph.D. thesis, University of Bristol, England, 1986).

The organism was also recovered from the vagina of four of eight females, three of which had been vaccinated. Onset of excretion varied from 9 to 25 days pc and continued for 21 days in three cats and until 159 days pc in the other (Fig. 1c, g, h, and j). Chlamydiae were isolated sporadically from rectal swabs of four cats, three of which had been vaccinated (Fig. 1d, f, g, and j).

**Chlamydial serology.** Vaccinated cats had IF antibody titers of 8 (one cat), 256 (two cats), and 512 (two cats) immediately before the challenge; these IF titers rose to \(>1,024\) by 8 weeks pc. IF titers of 512 to 2,048 developed in unvaccinated cats by 8 weeks pc.

Vaccination did not induce the formation of detectable CF antibodies in any of the five cats, but three of them devel-
opened low titers of CF antibodies after challenge. These CF antibodies were first detected at 1, 4, and 8 weeks pc for the three cats, and the titers were 40 (two cats) and 10 by 17 weeks pc. Two of five unvaccinated cats also developed CF antibodies (titer 10) by 8 and 17 weeks pc. The titer in the first of these cats had risen to 20 by 17 weeks pc.

**Isolation of viruses and bacteria.** Viruses were not isolated from any cat during the period of study. Bacterial cultures from conjunctival swabs taken 7 and 18 days pc revealed very scant growth of *Acinetobacter* spp. on three occasions and nonhemolytic *streptococci* on one occasion.

**DISCUSSION**

Conjunctival challenge with FPN produced clinical disease in unvaccinated cats which was characterized principally by conjunctivitis and ocular discharge and only minimally by respiratory signs. This confirms the findings of others that, despite its original association with pneumonitis (1), the FPN strain of *C. psittaci* is usually associated with conjunctivitis (10, 27). In contrast to an earlier description of feline *C. psittaci* infection (5), the cornea was not involved.

Vaccination with the modified live vaccine significantly reduced clinical disease following challenge. However, there was no effect on the number of organisms shed from the eye or on their ability to infect and persist at other sites. The ability of the vaccine to control clinical disease but not infection may be explained by postulating that (i) the acute ocular disease is caused largely by release of soluble toxic chlamydial products into the eye rather than as a direct cytopathic effect of chlamydial replication within the conjunctival mucosa, and (ii) vaccine-induced antibodies neutralize these toxins while having no effect on chlamydial infectivity or replication. Previous work has shown that...
during replication of other chlamydiae in vitro, chlamydial antigen could be detected at the surface and in the vicinity of infected cells (19). Yolk sac-propagated FPN is known to produce a toxin (7), which may be the immunodominant antigen of this chlamydial strain (13). Release of this toxin, lipopolysaccharide, or another unidentified antigen(s) onto the eye during FPN infection may contribute significantly to the pathogenesis of the ocular disease caused by this organism.

All but one vaccinated cat had developed high IF antibody titers to FPN by the time they were challenged. Future immunological analysis of FPN antigens with sera from these vaccinated cats by Western blot (immunoblot) or radioimmunoprecipitation techniques may therefore help to define important pathogenic components of this organism.

CF antibodies were not detectable in five of 10 (50%) of the cats 17 weeks pc, although they had IF antibody titers of 512 to 2,048. This suggests that the CF test is an unreliable serological test for chlamydial infection in cats.

An additional, unexpected finding of this study was the prolonged excretion of organisms that frequently occurred from the gastrointestinal and genital tracts after experimental ocular challenge with FPN. This was seen in both vaccinated and unvaccinated cats (Fig. 1). Persistent chlamydial infection in the presence of high levels of circulating antibodies is well recognized in chlamydial infections, since these antibodies have little neutralizing ability (15). The mode of transmission of chlamydiae to these extraocular sites, the exact anatomical sites involved in infection, and the significance of such infection in the production of clinical disease in the cat are not yet known. Hargis et al. (8) identified chlamydiae in the superficial gastric mucosa of cats; these organisms produced conjunctivitis and rhinitis when inoculated into specific-pathogen-free kittens (6). The stomach may therefore be a site of persistent FPN infection in cats. In a previous study, C. psittaci was recovered on one occasion from the genital tract of a female cat (5), but the present study is the first report of persistent genital tract infection. Moreover, this infection occurred as a result of ocular rather than direct genital tract inoculation. Persistent genital tract infection may be relevant to the suggestion that naturally occurring chlamydial infection in the female is a cause of reproductive failure in catteries (20, 27). However, the mechanism for such failure is not yet clear. Although salpingitis and adhesion formation occurred after FPN was inoculated directly into the oviducts of cats (11), these conditions are unlikely to be consequences of natural chlamydial infection, since the presence of valve systems between the cat uterus and oviducts is likely to inhibit the spread of infection beyond the uterus.

Our work has shown that FPN infection, like chlamydial infections in other species which are clinically manifested by ocular disease, is not restricted to the conjunctival mucosa. The contribution of extraocular FPN infection to the persistence and pathology of feline chlamydial disease is still largely unknown. Further vaccination studies should help to elucidate the pathogenesis of disease caused by this chlamydial agent.

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LITERATURE CITED