Ocular Involvement with Chlamydia psittaci (Strain M56) in Rabbits Inoculated Intravenously

J. O. Iversen, J. Spalatin,
C. E. O. Fraser and R. P. Hanson*

ABSTRACT

Fourteen albino rabbits were inoculated intravenously with $10^{3.5}$-$10^{4.0}$ mouse $ICLD_{50}$ of Chlamydia psittaci (strain M56) of mammalian origin. Ocular lesions accompanied the chlamydial infection in the rabbits. Bilateral anterior uveitis, a common occurrence, began on the second or third day and subsided by the tenth day whereas keratoconjunctivitis was observed infrequently. After 15 days the most prominent microscopic lesion was iritis. Accumulations of inflammatory cells, mainly plasma cells, were observed in the iris and ciliary body and elementary bodies were found infrequently in macrophages.

Chlamydiae were recovered consistently by conjunctival swabbing from the fifth to the twenty-fourth day. The agent was present within the eye (viz. iris-ciliary body) in three of four rabbits killed at 15 days and in five of ten rabbits killed 60 days after inoculation. Chlamydiae had persisted in the cerebrum and joints as well. Although neutralizing antibody was consistently present in sera at 60 days none of the samples of aqueous humor were capable of neutralizing the agent.

It is suggested that systemic chlamydial infections in the rabbit provide a model for the study of endogenous uveitis, a common ophthalmological problem.

RÉSUMÉ

L'injection intra-veineuse, à 14 lapins albinos, de $10^{3.5}$ à $10^{4.0}$ LD_{50} intra-cérébrales de souris de la souche M56 de Chlamydia psittaci, préalablement isolée chez des mammifères, provoqua l'apparition de lésions oculaires. Une uvéite antérieure bilatérale s'avéra fréquente, dès le deuxième ou le troisième jour après l'injection, mais elle disparut au bout de dix jours. Par ailleurs, on observa rarement de la kérato-conjonctivite. Au bout de 15 jours, l'inflammation de l'iris constituait la lésion microscopique prédominante. On décela des cellules inflammatoires, surtout des plasmocytes, dans l'iris et le corps cilié, mais rarement des corps élémentaires dans les macrophages.

Du cinquième au 24e jour après l'injection, on recouvra régulièrement C. psittaci dans des écouvillons de la conjonctive. Le microorganisme était présent dans les structures internes de l'oeil, v.g. l'iris et le corps cilié, chez trois des quatre lapins sacrifiés 15 jours après l'inoculation, ainsi que chez cinq des dix abattus 45 jours plus tard. L'agent avait également survécu au sein des hémisphères cérébraux et dans des articulations. Même si on décela de façon constante des anticorps sériques neutralisants, au bout de 60 jours, aucun des échantillons d'humeur aqueuse ne put neutraliser l'agent.

Il semble que la chlamydiose systémique du lapin fournisse un modèle pour l'étude de l'uvéite endogène, une condition ophtalmique fréquente.

INTRODUCTION

The role of many members of the genus *Chlamydia* (psittacosis-lymphogranuloma-trachoma group) (11) in infections involving the external ocular structures has been well established (8). However, scant attention has been paid to intraocular involvement with chlamydial agents. During studies of experimental infections in different animal species with a chlamydial

*Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan (Iversen) and the Department of Veterinary Science, University of Wisconsin, Madison, Wisc. (Spalatin, Fraser and Hanson).

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agent of mammalian origin, strain M56 (6, 15), three observations were made: first, regular involvement and persistence of this agent in the eyes of albino mice after systemic exposure, second, corneal opacity in a juvenile cottontail rabbit after intravenous inoculation and third, the persistence of the agent in the eyes of rabbits and hares surviving generalized infection. This paper describes pathological and microbiological findings from investigations on the involvement of ocular structures after systemic chlamydial infection in domestic rabbits.

MATERIALS AND METHODS

Chlamydial Strain

The agent used (strain M56) was isolated from muskrats (*Ondatra zibethicus*) in Saskatchewan, Canada, in 1961 (14). During the same year, similar isolates were obtained from snowshoe hares (*Lepus americanus*) in Saskatchewan. These organisms were placed in subgroup B of the genus *Chlamydia* (4, 15). After six passages via yolk sac inoculation in seven day old embryonated hens' eggs incubated at 37°C the infectivity titer reached $10^{7.5}$ median chick embryo lethal doses ($CELD_{50}$)/ml and $10^{8.5}$ median mouse intracerebral lethal doses (mouse $ICLD_{50}$)/ml in three week old albino mice.

Rabbits

Albino domestic rabbits (*Oryctolagus cuniculus*) were obtained from the Voss Rabbitry, Madison, Wisconsin. They were inoculated via the lateral ear vein with a 10% suspension of uninfected yolk sac material in saline (controls) or with a 10% suspension of strain M56 infected yolk sac material in saline.

Two experiments were conducted:

1. Four infected and two control rabbits were killed 15 days after inoculation with $10^{4.0}$ mouse $ICLD_{50}$ of strain M56 per animal (Table I). Rectal temperatures and clinical signs of ocular involvement were recorded daily. At the conclusion of the experiment ocular tissues were taken for histopathology and for chlamydial assay.

II. Ten infected and three control rabbits were killed 60 days after inoculation with $10^{3.5}$ mouse $ICLD_{50}$ of strain M56 per animal. During the course of the experiment conjunctivae were swabbed for chlamydial assay (Table II). Cotton swabs were moistened with tryptone broth, inserted under the eyelids, rotated vigorously over the surface of the palpebral and bulbar conjunctiva, placed in a test tube containing 0.5 ml of tryptone broth and rotated thoroughly against the side of the tube. The broth was then assayed for the presence of the agent. At the conclusion of the experiment the tissues of all rabbits were assayed for the presence of the agent (Table III). After removal of the eye from the orbit the proximal surface of the globe was seared with a hot spatula and the iris and ciliary body were removed via incisions through the seared surface. Swabs of the femoral tibial articulation were prepared with the same technic used for the conjunctival swabs. In addition sera and samples of aqueous humor were collected for neutralization tests.

TABLE I. Ocular Pathology after Experimental Systemic Infection with *C. psittaci* (strain M56) in Domestic Rabbits (Experiment I)

Exposure	Animal No	Recovery of Strain M56 from eye[a]	Ocular Pathology			
			Episcleral Injection	Ciliary Injection	Corneal Opacity	Microscopic Lesions[a]
Control.....	3	−	−	−	−	None
	4	−	−	−	−	None
Infected with strain M56	13	+	+	+	−	Iritis, synechia
	14	−	+	+	−	None
	15	+	+	+	−	None
	16	+	+	+	+	Iritis, keratitis

[a]The rabbits were killed 15 days after inoculation

TABLE II. Recovery of *C. psittaci* (strain M56) from Conjunctival Swabbing of Domestic Rabbits Infected Intravenously (Experiment II)

Exposure	Rabbit No	Days Post Infection 1	10	20	30	40	50	60
Controls	108			−[a]	−	−	−	−
	109			−	−	−	−	−
	110			−	−	−	−	−
Infected with strain M56	121			+	+	+	+	+
	122			+	−	−	−	−
	123			−	−	−	−	−
	124			−	−	−	−	−
	125			+	+	−	−	−
	126			+	−	−	−	−
	127		+	+	+	+	+	−
	128		+	+	+	+	+	−
	129		+	+	+	+	+	−
	130		+	+	+	+	+	−

[a] + = strain M56 recovered; − = strain M56 not recovered

Chlamydial Assay of Tissue and Swabs

The agent was assayed by intracerebral inoculation of three week old albino mice (strain Ha/ICR, A. R. Schmidt Co., Inc., Madison, Wisconsin) with 10% suspensions of tissues or with fluids obtained from conjunctival or joint swabs. After preparation of the suspensions four mice were inoculated immediately with each. These mice were examined daily for two weeks. Mean death times were calculated and the titers were estimated by the single-dilution method of Golub (5) adapted in our laboratory for intracerebral inoculation of three week old mice (6).

Serology

Neutralization tests were performed in three week old albino mice as previously described (6).

Histopathology

Eyes were fixed in buffered 10% formalin (pH 6.5) and imbedded in paraffin. Sections were stained with hematoxylin and eosin, Giemsa's and Noble's stains (9). Examination was made for lesions and intracytoplasmic inclusion bodies.

TABLE III. Titers of *C. psittaci* (strain M56) from Tissues[a] of Systemically Infected Domestic Rabbits 60 Days after Exposure (Experiment II)

Exposure	Rabbit No	Tissue: Internal Eye[b]	Brain	Joints
Controls				
	108	0[c]	0	0
	109	0	0	0
	110	0	0	0
Infected with strain M56	121	0	2.0	0
	122	2.3	4.0	0
	123	3.0	2.7	2.0
	124	0	0	0
	125	2.0	2.7	0
	126	0	0	0
	127	0	0	0.7
	128	0	1.0	0
	129	2.0	2.7	1.0
	130	1.0	2.5	1.0

[a] The agent was not detected in the blood, lung, liver-spleen, or kidney of any of the rabbits 60 days after exposure

[b] Internal Eye = iris and ciliary body

[c] Log_{10} mouse $ICLD_{50}$ per gram of tissue or per ml of fluid

RESULTS

Experiment I

Clinical Findings — The infected rabbits developed acute febrile illness. Rectal temperatures were elevated 1.1° to 2.2°C by the sixth day and returned to normal by the fifteenth day. Lassitude and marked emaciation were common characteristics terminally. Signs of anterior uveitis were easily observed in the unpigmented iris of the albino rabbits. Bilateral hyperemia of the iris was first observed two to three days after infection. On the fifth and sixth days there was a deep dull red vascular injection near the corneoscleral limbus. Edema and congestion of the iris led to a small pupil which reacted poorly to light. Conjunctivitis was apparent. By the tenth day, the vascular congestion had cleared from the anterior uvea and the conjunctiva. One domestic rabbit had unilateral corneal opacity on the seventh, eighth and ninth days.

Histopathology of the Eye — The microscopic lesion common to the involved eyes was an iritis (Table I). There were accumulations of plasma cells, lymphocytes and monocytes in the iris and ciliary bodies. Plasma cells predominated. Elementary bodies were found infrequently in macrophages. In one animal there was a marked thickening of the cornea characterized by edema, fibroblastic proliferation and leukocytic infiltration of the substantia propria in one eye. Posterior synechia occurred unilaterally in another animal.

Microbiological Findings — The agent was present in the intraocular tissues and conjunctival swabs of three of the four domestic rabbits killed on the fifteenth day after infection (Table I). Conjunctival swabs taken on the tenth day yielded the same pattern. The agent was also recovered from the brains and from liver-spleen combinations in all four of the infected animals.

Experiment II

Microbiological Findings — The agent was recovered from the conjunctiva beginning on the fourth day (Table II). Peak titers of 10^4 mouse $ICLD_{50}$ of agent were obtained from 14 to 16 days and the titers began to decrease after day 16. Chlamydiae were not recovered by swabbing from most of the rabbits by day 30 and the agent was recovered from only one of ten rabbits on day 60.

On the sixtieth day after infection, chlamydiae were found in the eye (iris and ciliary body) in five of the ten rabbits (Table III). In addition, the agent had persisted in the cerebrum and joints.

Serological Findings — Pre-inoculation sera were negative. After 60 days, sera from all the inoculated rabbits neutralized at least $10^{3.0}$ mouse $ICLD_{50}$ of the agent, whereas none of the samples of aqueous humor were capable of neutralizing the agent.

DISCUSSION

Chlamydiae producing systemic infections should be considered in ocular syndromes of unidentified etiology. Following experimental systemic infections ocular disease resulted from the presence of chlamydiae (strain M56) in the eyes. The anterior uveitis that was observed is comparable to the uveitis associated with lymphogranuloma venereum, a chlamydial infection of man (7). In addition to the iris and ciliary body, strain M56 produced lesions in the cornea and conjunctiva. Numerous chlamydial agents appear to be involved in syndromes of the superficial ocular tissues in a variety of mammals *e.g.* inclusion conjunctivitis of guinea pigs (10), catarrhal conjunctivitis of cats (2), keratoconjunctivitis of sheep (2, 3, 8), an ocular syndrome in piglets (8) and the classical trachoma of man (8). Trachoma infections are considered to involve the superficial ocular structures only.

Conjoint persistence of chlamydial agents in ocular tissues and other organs is not unique to strain M56. Since the earliest work with psittacosis it has been known that the agent induces conjunctivitis in mice infected by the intracerebral route (8). Bovine abortion due to a chlamydial agent was accompanied by keratoconjunctivitis and a characteristic hepatopathy in the fetuses (16). In experiments done by Storz and his coworkers sheep inoculated intra-peritoneally with the sheep polyar-

thritis agent developed follicular conjunctivitis three days later and inclusions could be demonstrated in conjunctival smears (8). In Reiter's syndrome of man chlamydiae have been isolated from the conjunctiva, the synovial membranes and the urethra (13).

Persistence in the eyes following generalized infections with chlamydiae could afford another favorable site for the establishment of the latent infections characteristic of these organisms. Presumably the intra-cellular location of the chlamydiae protects the agent from the humoral antibodies. In view of the persistence of strain M56 in the eye in significant titer it might be useful to sample both intraocular and superficial ocular tissues and fluids during epizootiological surveys for chlamydiae in domestic and wild animals.

The ocular persistence of *Chlamydia psittaci* (strain M56) following systemic infection of rabbits provides an experimental model for the study of endogenous uveitis, a major ophthalmological problem (1). Endogenous inflammations may arise hematogenously and because of their common blood supply inflammations of the iris, the ciliary body and the choroid tend to be involved in the same inflammatory process. Following the experimental systemic infections endogenous uveitis was associated with the persistence of strain M56 in the iris and ciliary body. Three mechanisms could be tested to determine the basis of the pathological changes induced by the chlamydiae: 1) a direct effect due to the proliferation of the agent, 2) an allergic mechanism and 3) the toxic effect of the agent. Concerning the latter point, all known members of the genus *Chlamydia* possess toxic properties capable of damaging the vascular epithelium (12).

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