Chronic Ocular *Chlamydia trachomatis* Infection in Rabbits: Clinical and Histopathological Findings in the Posterior Segment

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Submitted: October 11, 2013
Accepted: January 16, 2014

Citation: Boiko EV, Pozniak AL, Maltsev DS, Suetov AA, Nuralova IV. Chronic ocular *Chlamydia trachomatis* infection in rabbits: clinical and histopathological findings in the posterior segment. *Invest Ophthalmol Vis Sci*. 2014;55:1176–1183. DOI: 10.1167/iovs.13-13416

Purpose. To investigate clinical and histopathologic manifestations of *Chlamydia trachomatis* (CT)-induced chronic posterior segment (PS) inflammation in rabbits.

Methods. Fifteen rabbits were divided into three equal groups of CT subconjunctival-only (SC) and subconjunctival plus intravitreal (SC+IV) inoculation, and controls. Both noncontrol groups received a bilateral SC injection (BSI) and the SC+IV group additionally received a unilateral IV injection (UII) of CT L2 culture, whereas the controls received a BSI+UII of phosphate-buffered saline. During 6 months post injection, the animals were investigated for PS inflammation and infection clinically and microbiologically (cell culture, ELISA, and real-time PCR). Hematoxylin-eosin staining and direct immunofluorescence in situ reaction were used to reveal the signs of tissue inflammation and infection.

Results. In the SC group, mild PS disorders (eight eyes) involving vitreal infiltration, the following posterior vitreous detachment and chorioretinitis, and severe PS disorders (two eyes) in the form of panuveitis, were developed. In the SC+IV group, mild (three and three eyes that received SC-only and SC+IV injections, respectively) and severe (two and two eyes that received SC-only and SC+IV injections, respectively) PS disorders were developed. A high titer (1:32–1:128) of CT-specific IgM antibody was present in sera from all the noncontrol animals. The CT antigen was detected in the conjunctiva and PS structures (the vitreous, retinal pigment epithelium, and choroid) in 100% and 40% to 75% of all the noncontrol animals, respectively.

Conclusions. Conjunctival or intraocular inoculation with CT may result in invasion of the PS structures and durable persistence thereof, with the development of inflammatory and then degenerative changes. These data might advocate for expanding the role of chronic CT infection in etiology and pathogenesis of vitreoretinal disorders.

Keywords: *Chlamydia trachomatis*, retina, retinal pigment epithelium, vitreous humor, chorioretinitis

It is well known that *Chlamydia trachomatis* (CT) are actual infectious agents in anterior segment[1–3] and urogenital diseases,[4] and induce chronic inflammation which has a major role in their pathogenesis.[4] Screening results have shown rather high prevalences (3.9%–22.1%) of this obligate pathogen in various populations,[5–7] with bacteremia incidence approaching 90%,[8] which suggests low-grade chronic inflammatory involvement of various organs with minimal symptomatology.

Dissemination of the agent has been confirmed by the CT-induced ocular (and, particularly, conjunctival) damage reported in chronic genital chlamydial infections, where the agent localization has been proved by various methods.[9] Besides, CT is known to be a trigger of anterior uveitis in genetically predisposed patients.[2,3] However, the works on CT-induced posterior segment (PS) damage are few and describe only uveitis,[10] chorioretinitis,[11] and endophthalmitis,[12] with pathogenesis, histopathologic features, and localization of the infectious agent in this damage remaining unclear. The available data on topical localization are limited to detection of the agent in subretinal fluid in rhegmatogenous retinal detachment that has been found in our study in 74% of the patients,[13] and confirmed by Ghaffariyeh et al.[14] in one patient. Hence, the details of PS damage remain poorly investigated, and modeling and studying chronic chlamydial infection of this localization is deemed to be required.

Materials and Methods

Animals

A total of 15 Chinchilla adult male rabbits (age, 12–14 months and weight, 2.5–3 kg) were used in the study. All these had normal findings on physical and ophthalmic examinations, and were free of serum antichlamydial antibodies. Experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and approved by a local Ethics Committee of the Military Medical Academy (MMA).

Inoculation of CT

Chlamydia trachomatis serovar L2 (obtained from culture bank of MMA) was passaged on McCoy cell culture; bacterial cells were purified and concentrated by the technique of

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Campbell et al.[15] The rabbits were divided into three equal groups. Groups of CT subconjunctival (SC)-only ($n = 5$) and SC+intravitreal (SC+IV, $n = 5$) inoculation received a bilateral SC injection of 4×10^5 inclusion forming units (IFU) of CT L2 in 0.1 mL of PBS. Moreover, the SC+IV group received an IV injection of 2×10^5 IFU of CT L2 in 0.05 mL of PBS to the right eye. The control group ($n = 5$) received a 0.1-mL bilateral SC injection of PBS and a 0.05-mL IV injection of PBS to the right eye.

All IV injections were performed with a 30-gauge needle under sterile conditions and by direct visualization, and were made into the central vitreous, avoiding the lens. Before IV injection, animals were anesthetized with pentobarbital sodium (30 mg/kg, intravenously, supplemented as needed).

Clinical Examination

Clinical examination was performed daily for 3 weeks after inoculation, then thrice a week until the end of the experiment, and involved biomicroscopy, indirect ophthalmoscopy with SL-45 slit-lamp (Shin Nippon, Tokyo, Japan), image registering with TRC-50DX fundus camera (Topcon, Tokyo, Japan), and ultrasonography with US-3300 Echo Scan (Nidek, Tokyo, Japan). Pupils were dilated with instillation of 1% tropicamide (Mydriacyl; Alcon-Couvreur, Puurs, Belgium) before ophthalmoscopy.

Samples Collection

Samples were collected before inoculation and on day 7 postinoculation (PI). In all the animals, blood and conjunctival swabs were taken from the auricular vein and both eyes, respectively.

A portion of the blood collected for bacterial culture was used for serum preparation to measure the levels of IgM antibodies against CT. Antibody titers were determined with ELISA. The serum samples obtained from noninfected rabbits were used as negative controls. The conjunctiva was anesthetized with proxymetacaine 0.5% eye drops (Alcaine; Alcon-Couvreur) before swabbing.

Once a portion of the material obtained from a conjunctival swab was spread on a slide, the excess material was placed into universal transport medium (UTM; COPAN, Murrieta, CA) for culture examination and PCR.

ELISA Test

Serum dilutions were added to *C. trachomatis* IgM ELISA Kit (GenWay, San Diego, CA) microplate wells coated with purified antigen. Goat anti-rabbit IgM mu chain (horseradish peroxidase) secondary antibodies (Abcam, Cambridge, UK) were used to detect the antigen/primary antibody complex. Excess conjugate was washed out and tetramethyl benzidine substrate (from the ELISA kit) was added. The reaction was stopped by adding 2 M H_2SO_4. Optical density values were read in an Immunochem-2100 (HTI, Walpole, MA) microplate reader at 450 nm (reference wavelength, 630 nm).

Direct Immunofluorescence Assay (DFA)

The DFA is based on binding of antibodies to an epitope, a specific trisaccharide component αKdo(2→8)αKdo(2→4)αKdo of cell wall lipopolysaccharide (LPS). The relatively high thermal stability of LPS provided for identification of CT in histologic specimens. Dewaxed histologic specimens, conjunctival swabs, and cell cultures were incubated in the presence of fluorescein-conjugated monoclonal antibodies (ChlamyScan; LABDiagnostica, Moscow, Russia) for 20 minutes in a moisture chamber.

After being washed in PBS (pH 7.2) for 10 minutes, the cell culture specimens were dried and coverslipped with 10% glycerin solution in PBS. A sample was considered CT-positive if at least four elementary bodies or any CT inclusions were detected.

Cell Culture

McCoy cells were cultured in 24-well cell culture plates with 5% fetal bovine serum-supplemented Eagle's minimum essential medium. Pretreated with 1 mg/L cycloheximide (Acros Organics, Geel, Belgium) cultures were inoculated with 0.2 mL of each transport medium specimen or blood sample. Microplates were incubated at 37°C for 96 hours, washed with PBS, fixed in methanol, and subjected to DFA.

PCR Testing

The DNA was extracted from conjunctival swab specimens using DNA-sorb-B Nucleic Acid Extraction kit (AmpliSens, Moscow, Russia), according to the manufacturer's instructions. Specimens were tested for the presence of DNA from CT using *C. trachomatis*-screen-titer-FRT (AmpliSens). Real-time PCR was performed on a rotary analyzer Rotor-Gene 6000 (Corbett Research, Sydney, Australia). The PCR results were interpreted according to the manufacturer's instructions.

Histopathology

The rabbits were euthanized by 100 mg/kg intravenous injection of pentobarbital on day 189 PI. Enucleated globes were fixed in 10% neutral-buffered formaldehyde and embedded in paraffin. Serial sections (4–6 μm thick) were cut, deparaffinized, and stained with hematoxylin and eosin (H&E). Histologic evaluations of conjunctival, retinal, and choroidal tissues were performed by light microscopy. At least four sections of each eye, with at least one of these sections having conjunctival and chorioretinal changes, were subjected to DFA.

Statistics

Statistical analysis was performed with Statistica 6.0 software (StatSoft, Inc., Tulsa, OK) using 1-tailed Fisher's exact test for comparison of qualitative data. P values below 0.05 were considered statistically significant.

Results

Clinical Findings

The animals remained in good general health. However, as for eye disorders, marked variation was found in the severity of the damage to the posterior segment (PSD), which generally may be divided into mild and severe.

Mild PSD (Chorioretinitis). The rabbits developed conjunctivitis with moderate vascular injection and insignificant amount of discharge on days 2 to 4 PI (Fig. 1, left) and progressed to chronic conjunctivitis persisting to the end of the experiment. On days 4 to 7 PI, they developed preretinal infiltration into the vitreous (Fig. 1, right) that usually resolved slowly by the end of month 2 PI. However, in some cases residual infiltration persisted to the end of the study. On days 10 to 16 PI, examination revealed yellow-white foci slightly extending from the choroid into the vitreous body, with retinal opacity and inflammatory vitreous infiltrate above them (Fig. 2, left). Resolution of foci on days 27 to 45 PI was accompanied

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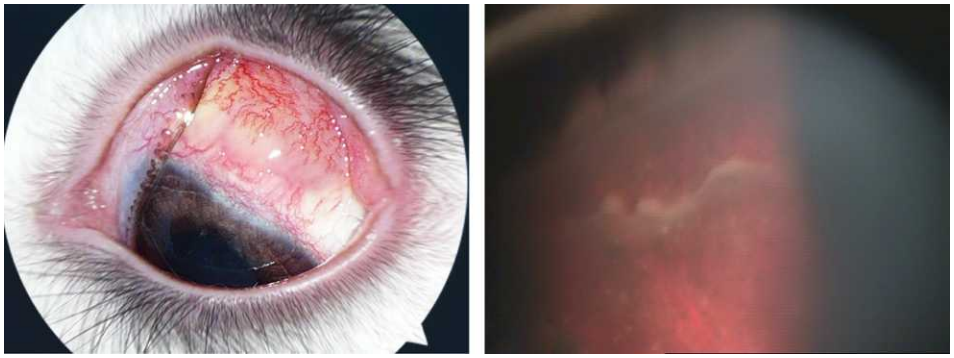

FIGURE 1. Rabbit's left-eye anterior segment (*left*) shows conjunctivitis with moderate vascular injection and fundus (*right*) shows infiltration in the preretinal vitreous at day 11 after SC-only inoculation of the eye with CT (SC+IV group).

by retinal clarity restoration, decrease in the size, flattening and sharpening of the outlines of foci, and pigmentary changes. These events were concurrent with the decrease in the amount of inflammatory vitreous infiltrate (Fig. 2, right).

Severe PSD (Panuveitis). Severe damages were observed in three rabbits (six eyes) which developed conjunctivitis with marked vascular injection, conjunctival edema, and mucous purulent discharge as early as day 3 PI. Transition to the chronic form of conjunctivitis was concurrent with the development of follicles in conjunctival fornices. In these three rabbits, the development of panuveitis was observed, manifested as iris edema, marked inflammatory infiltrate into the anterior chamber aqueous humor, and early (day 3 PI) appearance and fast increase in the amount of inflammatory vitreous infiltrate. Subsequently, lenticular and corneal opacity (Fig. 3, left) was observed progressing to the level at which ophthalmoscopy was impossible and persisting to the end of the experiment. Ultrasonography revealed exudative retinal detachment and vitreous opacities (Fig. 3, right) by day 21 PI. The three rabbits with severe PSD involved two rabbits (four eyes) and one rabbit (two eyes) of SC+IV and SC groups, respectively. However, in another three rabbits of the SC+IV group, mild PSD was observed.

Nevertheless, in each animal of the noncontrol groups, regardless of the severity of PSD and despite unilateral pattern of IV injections (if any) performed, clinical manifestations were similar in both eyes (difference between the left and right eyes, $P = 0.678$). No statistically significant difference was found in the severity of the infection process between the noncontrol groups ($P = 0.529$). At the end of experiment, as regards the noncontrol groups, slight conjunctivitis ($n = 5$), signs of neither conjunctivitis nor inflammation process in the PS ($n = 2$), and marked conjunctivitis combined with panuveitis ($n = 3$) were observed. In two and three of five slight conjunctivitis cases, mild signs of PS inflammation and no obvious PS pathology were observed, respectively. In the control group, changes in the ocular structures were detected ophthalmoscopically neither throughout the follow-up period nor at the end of the experiment.

Results of the Microbiological Examination and Serodiagnosis

In each animal of the noncontrol groups, CT was detected based on the results of at least two assay techniques from those mentioned in Table 1, with the conjunctival swab cultures (DFA) technique showing the highest number of detection events. In all the animals, the antibodies were detected at different titers (Table 1).

Histologic Findings

Conjunctiva. Inflammatory infiltration of the conjunctiva (lymphocytic inflammatory infiltrate) was detected in mild and severe cases (Table 2). In severe cases, lymphocyte clusters were revealed (without any signs of proliferation of inflammatory cells in the subepithelial connective tissue) which resulted in extension of the conjunctiva into conjunctival cavity.

Vitreous. In mild cases (Table 2), lymphocytic inflammatory infiltrates were revealed mostly in the preretinal layers and posterior hyaloid membrane (PHM). A PHM detachment (Fig. 4A) was seen in the presence of areas of residual fixation due to accumulation of inflammatory infiltrate penetrating the neuroepithelium and gliosis (Fig. 4B). In panuveitis, histopa-

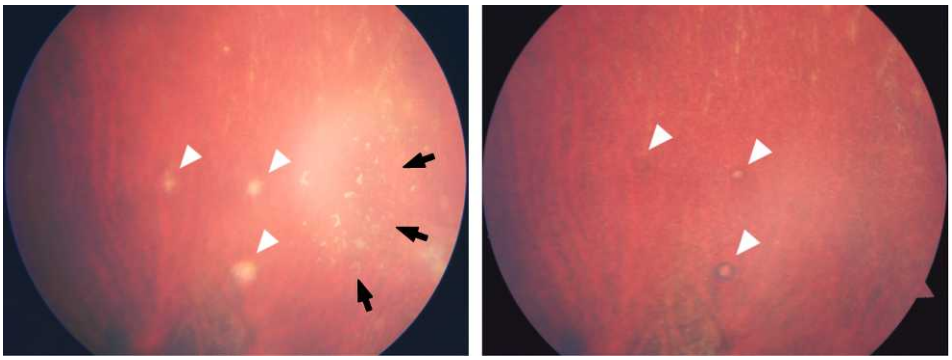

FIGURE 2. Rabbit's left-eye fundus after SC-only inoculation of the eye with CT (SC+IV group). Active chorioretinal lesions (*arrowheads*) and inflammatory vitreous infiltrate (*arrows*) (*left*) in the fundus periphery at day 11 PI and atrophic chorioretinal lesions (*arrowheads*) in absence of inflammatory vitreous infiltrate (*right*) at day 45 PI.

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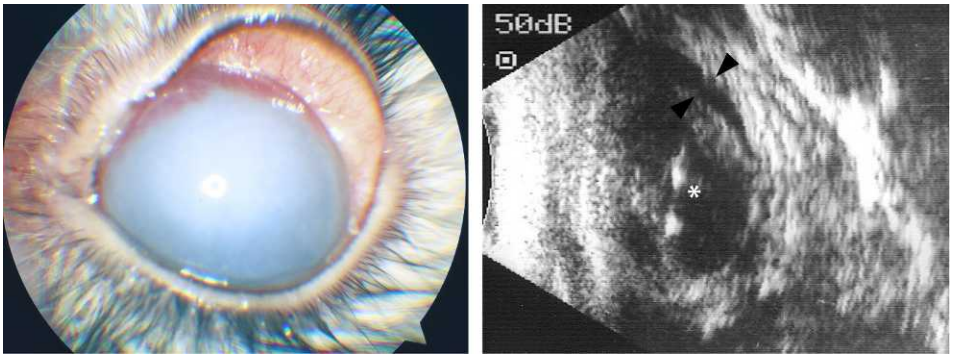

FIGURE 3. Rabbit's left-eye anterior segment (*left*) shows conjunctivitis, inflammatory exudate into the anterior chamber aqueous humor and corneal opacities, ultrasonography (*right*) shows exudative retinal detachment (*arrowheads*) and vitreous opacities (*asterisk*) at day 45 after SC-only inoculation of the eye with CT (SC+IV group).

thology confirmed the severe destruction of the vitreous body (Fig. 5A) observed on ultrasonography, with total detachment of and marked inflammatory infiltrate in the collapsed vitreous.

Retina. Foci of chorioretinal atrophy (Table 2) without signs of active inflammation were observed mostly in inner retinal layers, along with a diffuse inflammatory infiltrate of isolated cells. The foci represented the definitely outlined areas of retinal thinning resulted from loss of outer and inner layers of the neuroepithelium, and featuring direct contact of the neuroepithelium with Bruch's membrane, choroidal atrophy, PHM detachment, and absence of the RPE (Fig. 4C). In panuveitis (Table 2), exudative retinal detachment (Fig. 5A), retinal vasculitis (Fig. 5B), retinal folding, and vacuolization of the photoreceptor layer (Fig. 5C) were observed. Only isolated inflammatory cells were present at the bases of the folds, in four cases along with exudative retinal detachment and

TABLE 1. Results of Identification of *C. trachomatis* by DFA and PCR in Specimens and Determination of the Serum IgM Antibody Titer

	Inoculation										
	SC-Only					SC+IV					
Animals	1	2	3	4	5*	6*	7*	8	9	10	Total, *n* (%)
DFA, swabs	+	L	L	R	+	+	+	+	+	+	17 (85%)
DFA, swab cultures	+	L	+	+	+	+	+	L	+	+	18 (90%)
DFA, blood cultures	+	+	–	–	+	+	–	+	–	+	12 (60%)
PCR, swabs	+	L	R	–	+	+	+	+	+	–	14 (70%)
Antibody dilution	1:32	1:32	1:64	1:64	1:128	1:128	1:64	1:64	1:32	1:32	

R, detection in the right eye only; L, detection in the left eye only; +, bilateral detection; –, no detection.
* Reflects severe PSD.

TABLE 2. Results of Histopathology and DFA for *C. trachomatis*

	Inoculation										
	SC-Only					SC+IV					
Animals	1	2	3	4	5*	6*	7*	8	9	10	Total, *n* (%)
Histologic features											
Conjunctival inflammation	+	+	+	+	+	+	+	+	+	+	20 (100%)
Vitreous exudate	+	L	R	R	+	+	+	+	+	+	17 (85%)
Vitreous traction	+	+	–	–	+	+	+	R	L	+	14 (70%)
Vasculitis	–	–	–	–	+	+	+	+	–	–	8 (40%)
Retinal inflammation	+	L	R	R	+	+	+	+	+	+	17 (85%)
Retinal folding	–	–	–	–	+	+	+	–	–	–	6 (30%)
Chorioretinal lesions	+	–	–	+	–	–	–	+	+	+	10 (50%)
DFA detection of *C. trachomatis* antigen											
Conjunctiva	+	+	+	+	+	+	+	+	+	+	20 (100%)
Vitreous	–	–	–	–	+	+	+	+	–	–	8 (40%)
Retina	R	L	R	R	+	+	+	+	L	+	15 (75%)
Pigment epithelium	R	–	–	–	+	+	+	L	–	R	9 (45%)
Choroid	+	R	–	–	+	+	+	R	L	R	13 (65%)

* Reflects severe PSD.

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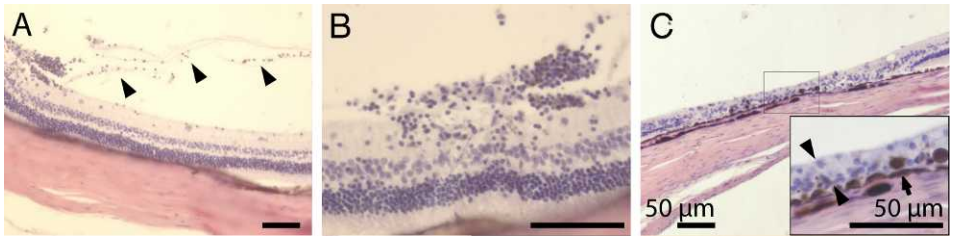

FIGURE 4. Histology micrographs with H&E staining at day 189 PI. Posterior hyaloid membrane detachment (*arrowheads*) (**A**) and local firm vitreoretinal adhesion (**B**) in the eye of the CT SC-only inoculated animal (SC group); and degenerative vitreoretinal lesion (*arrowheads*, neuroepithelum; *arrow*, RPE) (**C**) in the rabbit's right eye subjected to SC and IV inoculation with CT (SC+IV group). *Scale bars*: 50 μm.

dystrophic RPE changes (cell degeneration and loss of pigment granules).

RPE and Choroid. The displacement of 3- to 12-μm anuclear pigment clusters toward inner neuroepithelial layers was observed. Hypertrophy of individual cells involved the increase in cell height and pigment density. In panuveitis, RPE degeneration was characterized by spacious sites of flattened cells with residual amount of pigment seen in the projection of detached retina. The choroid had a slight inflammatory infiltrate even in severe uveitis. No presence of any other (bacterial) infection that could induce intraocular inflammation was registered.

DFA Findings

As one can see in Table 2 and the figures, the CT antigen was detected in the conjunctiva (Fig. 6, left), choroid (Fig. 6, right), vitreous body, different retinal (mostly inner) layers (Fig. 7, left), and RPE (Fig. 7, right). Pseudofollicular conjunctival structures always contained the antigen. The amount of detected antigen was not related to the amount of infiltrate. The infectious agent was not detected in and around atrophic chorioretinal foci. No preferential clumping of the CT antigen in retinal folding was detected. In exudative retinal detachment, the RPE had a relatively high content of the CT antigen.

DISCUSSION

Oh and Tarizzo[16] have demonstrated damage to the anterior segment structures (including rabbit's corneal endothelium) induced by CT; however, without identification of the infectious agent in the tissues. In the present work, we showed, for the first time to the best of our knowledge, clinical and histopathologic alterations in, namely, the PS, and proved the possibility of the CT-induced direct and indirect damage to the vitreous, retina, RPE, and choroid. Marked variability in clinical presentations of PSD was consistent with that described in other experimental models,[17] and in most cases mild and moderate chorioretinitis without specific manifestations was observed. The severity of clinical manifestations depended neither on inoculation technique nor whether 4×10^5 or 6×10^5 IFU of CT were used. In humans, mostly severe Chlamydia-induced PSD (uveitis, endophthalmitis) has been described.[11,12] However, by analogy with genital chlamydial infections, the degrees of severity of infection could be widely variable (from mild to severe).[7] This suggests the availability of mild and subclinical cases of infection in humans, and their number may be rather significant due to high prevalences of chlamydial infections,[5–7] but they are not clinically manifested and diagnosed.[7] In our experiment, this suggestion was confirmed by the following observation: in some cases, long after manifestation of the mild or moderately severe processes, signs of the active inflammatory process were not found clinically ("normal appearing eye"). Part of the reason for marked variations in the course of infection is polymorphism of the immune response genes.[18]

The fact that, regardless of the route of inoculation and dose of the infectious agent, the process of varying degree of severity was developed not in a single eye, but in both eyes of an animal, may be explained by individual sensitivity. Thus, panuveitis could develop following nonintravitreous inoculation, whereas SC+IV inoculation of a larger dose of CT did not always result in marked intraocular inflammation.

On the other hand, inhibition of apoptosis, modulation of transcription factors, and CT transitions to a persistent form CT[19] determine the tendency of the CT-induced diseases for chronic subclinical course.[7] Clinical observations of the development of infection process were confirmed by the results of microbiological examination (refer to Table 1). However, CT-negative results of some samples by some assay techniques (less than 4 loci of specific fluorescence in DFA), even in the case of frank infection manifested by rising antibody titer and presence of inflammatory PS changes, are indicative of difficulty in diagnosing the CT infection, which has been widely debated lately.[20] We used the soft approach (four loci of specific fluorescence) recommended by the manufacturer to evaluate DFA specimens, and this is the limitation of our study, because some researchers consider a sample positive if 10 loci of specific fluorescence are

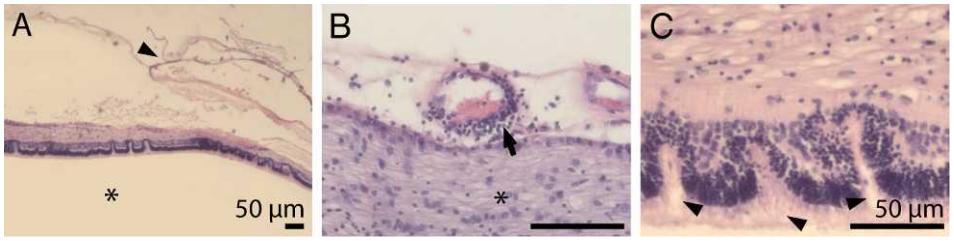

FIGURE 5. Histology micrographs with H&E staining at day 189 PI. Severe destruction of vitreous body (*arrowhead*) and exudative retinal detachment (*asterisk*) (**A**), vasculitis (*asterisk*, nerve fiber layer; *arrow*, vasculitis) (**B**) and retinal folding (*arrowheads*, vacuolization of photoreceptor layer) (**C**) in the rabbit's right eye subjected to SC and IV inoculation with CT (SC+IV group). *Scale bars*: 50 μm.

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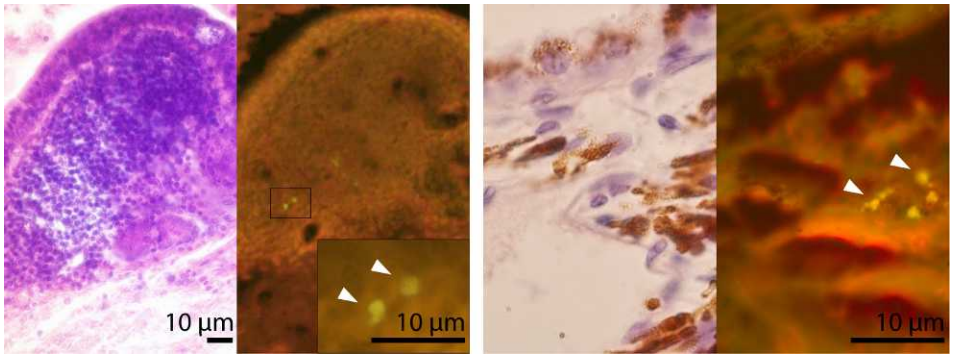

FIGURE 6. Histology micrographs with H&E and DFA staining of the rabbit's right eye (SC+IV group) at day 189 after SC and IV inoculation of the eye with CT. *Left*: antigen of *C. trachomatis* (*arrowheads*) in the conjunctiva. *Right*: antigen of *C. trachomatis* (*arrowheads*) in the choroid. *Scale bars*: 10 µm.

identified.[21] Therefore, to avoid false-positive results, the presence of CT infection, if any, was validated by PCR and serologically. There was a common pattern that follows the general principles of PS inflammation: cellular infiltration of the vitreous and retina, retinochoroidal lesions, and vasculitis. Attention is drawn to the detection of the infectious agent being an intracellular parasite in the vitreous, which already has been shown in endophthalmitis.[12] We speculated that this results from dissemination of the agent either from the adjacent cellular structures (including neuroepithelium) or with migrating inflammatory cells, whereas accompanying changes in the vitreous structure (condensation of collagen, liquification, and PHM detachment) result from inflammation process in the PS that already have been described[22] in other infectious PSD. In humans, chorioretinal foci presumably induced by CT have been described,[11] but, in our work, direct evidence of its ability to induce chorioretinitis was obtained. Furthermore, we showed that, in chronic phase of the disease, in and around these foci, the inflammatory changes and infectious agent are absent, unlike the vitreal changes, making the histologic picture similar to that of foci of degeneration.[23] Damage to the RPE undoubtedly is an important link in the pathogenesis of the infection-mediated PSD.[24] The evidence of direct CT infection of the RPE cells suggests that this infectious agent may have a role in the development of fundus pathology. The folding detected in our study has been described in a number of works on experimental autoimmune uveoretinitis,[25–27] and, therefore, might be a manifestation of an autoimmune process. On the other hand, such changes have been described by Hay and Dutton[28] in a murine model of congenital ocular toxoplasmosis, but also in the absence of the infectious agent, which does not exclude the possibility of autoimmune damage. Furthermore, a number of researchers draw attention to rarity of detection of infectious agents in various experimental models of PSD despite presence of inflammatory changes,[29] which may be explained by autoimmune or hypersensitivity reactions[30] and late examinations.[29] The opinion of Nussenblat et al.[31] on a key role of the autoimmune component in infectious uveitis conforms to these statements. This holds true particularly for CT, which is known to be a trigger of autoimmune processes in anterior uveitis,[3] and might have a similar role in PSD. In SC inoculation, CT easily disseminated to the PS, possibly due to its high potential for local and hematogenous dissemination,[8,32] with this fact being confirmed by histopathologic findings and CT-positive blood culture results. However, based on the present study, it is difficult to give priority either to local or to hematogenous dissemination associated with local inflammatory process and breakdown of the blood–aqueous barrier, respectively. In our experiment, high bacterial doses were administered, suggesting high probability of the agent dissemination.

Although it would have been interesting to inoculate smaller CT doses to determine the minimum infective dose, this was not the aim of the study.

Nevertheless, one may suggest that, in the natural course of CT infection in humans, the possibility of PS infection also exists. Identification of the infectious agent in the RPE, neuroepithelium, choroid, and vitreous is an evidence of its tropisms not only for various epithelial cells, but for those of other tissues, including neural tissue. These results are consistent with identification of nonspecific ligands for attachment of elementary bodies to eukaryotic cells,[33] and descriptions of experimental infection of lungs, liver, and

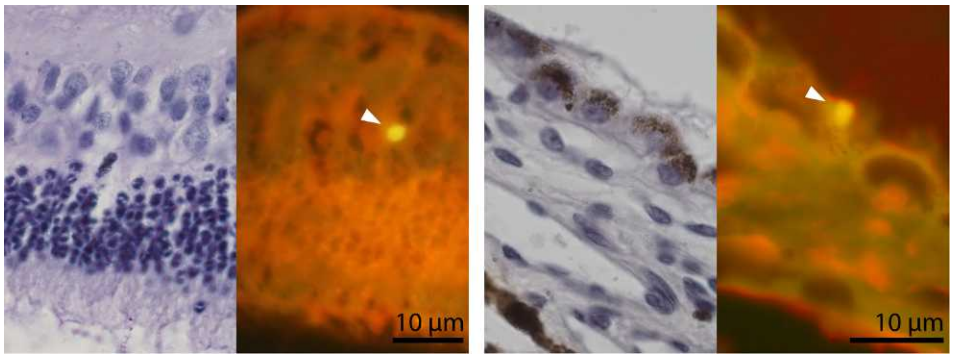

FIGURE 7. Histology micrographs with H&E and DFA staining of the rabbit's right eye (SC+IV group) at day 189 after SC and IV inoculation of the eye with CT. *Left*: antigen of *C. trachomatis* (*arrowhead*) in the retina. *Right*: antigen of *C. trachomatis* (*arrowhead*) in the RPE. *Scale bars*: 10 µm.

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spleen with CT,[34] perinatal chlamydial infections in humans,[35] and meningoencephalitis cases in humans.[36] In CT infection, pathogenetic mechanisms are implemented through antigenic stimulation, which, on the one hand, enhances the inflammation targeted against the infectious agent, and, on the other hand, is a prerequisite for the development of autoimmunity.[37] This mechanism takes on great significance because of long-term CT-specific persistence of the infectious agent in tissues,[37] which was confirmed for PS structures in the present study. Therefore, all the prerequisites for implementation of typical pathogenetic mechanism for the development of CT infection in the PS structures are present. Inflammatory processes have been considered to be critical in vitreoretinal diseases.[38,39] When taken together, the sites of firm vitreous attachment with vitreous traction, and atrophic neuroepithelial changes found in our study are a significant prerequisite to the development of retinal tears.

Like all experimental models, ours has limitations when extrapolated to humans. Other limitations of this study include the small group size and that a single time point was used for histopathology. Usually, animal models of the infectious PSD have another morphologic and clinical picture than that of the natural course of disease in humans.[17,29] Although the clinical importance of overt CT-induced PSD has been rather well known,[11,12] in our opinion, of special interest are the results related to subclinical CT-induced PS inflammation. Presence of the infectious agent in PS structures without clinical manifestations, but with histopathologic evidence of chronic low-grade inflammation, makes us consider the possible association of the pathogenesis of some vitreoretinal disorders with chronic CT infection. In the future, our findings can provide new insights regarding the biological mechanism of the disease and the design of a therapeutic strategy.

Acknowledgments

The authors thank Oleksandr V. Oleksiienko for his assistance in translating the article.

Disclosure: **E.V. Boiko**, None; **A.L. Pozniak**, None; **D.S. Maltsev**, None; **A.A. Suetov**, None; **I.V. Nuralova**, None

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