Institut Pasteur
Microbes and Infection 18 (2016) 254–262
www.elsevier.com/locate/micinf

Original article

# Infectious dose and repeated infections are key factors influencing immune response characteristics in guinea pig ocular chlamydial infection

Sandra Belij-Rammerstorfer [a], Aleksandra Inic-Kanada [a], Marijana Stojanovic [b], Emilija Marinkovic [b], Ivana Lukic [b], Elisabeth Stein [a], Jacqueline Montanaro [a], Nora Bintner [a], Nadine Schürer [a], Ehsan Ghasemian [a], Michael Kundi [c], Talin Barisani-Asenbauer [a,*]

[a] *OCUVAC – Center of Ocular Inflammation and Infection, Laura Bassi Centers of Expertise, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria*
[b] *Department of Research and Development, Institute of Virology, Vaccines and Sera – TORLAK, Belgrade, Serbia*
[c] *Institute of Environmental Health, Center for Public Health, Medical University of Vienna, Vienna, Austria*

Received 15 October 2015; accepted 7 December 2015
Available online 17 December 2015

**Abstract**

The aim of this study was to determine whether infectious dose of *Chlamydia caviae* after repeated infections influences the immunological responses and subsequent clearance of pathogen at the ocular surface of guinea pigs. Animals were infected three times via the conjunctiva at six- and twelve-week intervals by applying either $1 \times 10^4$ or $1 \times 10^6$ inclusion-forming units (IFUs) of *C. caviae*. Ocular pathology, infection course, *C. caviae*-specific serum IgG levels and their capacity to bind and neutralize infection *ex vivo* were assessed. Animals infected with $1 \times 10^4$ IFUs had completely diminished ocular infection and pathology after the 2nd infection with increased levels of *C. caviae*-specific serum IgG and their effective capacity to bind and neutralize *C. caviae*. Only partial protection was observed in animals infected with $1 \times 10^6$ IFUs after the 2nd and 3rd infections. Our findings show that full protection was observed in animals repeatedly infected with the lower dose. The lower dose appeared not to compromise the host immune system, thereby enabling fast clearance of the pathogen and the establishment of competent neutralizing antibodies.


*Keywords:* Trachoma; Infectious dose; *Chlamydia caviae*; Guinea pig ocular animal model

## 1. Introduction

Blinding trachoma belongs to the group of neglected tropical diseases. It is the primary cause of preventable blindness of infectious origin [1] and, according to current opinion, it is the end-stage of an ocular surface disease caused by repeated infections with *Chlamydia trachomatis* serovars A–C [2]. Re-infections are very common in endemic communities, as suggested by numbers of individuals who have repeated bouts of active trachoma [3]; the resulting chronic inflammatory response can subsequently lead to tissue remodeling and scarring. However, not all infected individuals develop blinding sequels like trichiasis and corneal opacity [4].

Cross-sectional studies show that chlamydial prevalence, organism load and ocular signs of active infection in endemic regions decrease with age, supporting the concept that protective immunity develops with time [5]. It has also been proposed that complete immunity is short-lived, as individuals can be reinfected and some display clinical signs. Factors contributing to the different outcomes and protection are not fully understood [2].

Experiments in animals can shed light on ocular chlamydial infection dynamics and the role of different

* Corresponding author. OCUVAC – Centre of Ocular Inflammation and Infection, Laura Bassi Centres of Expertise, Centre of Pathophysiology, Infectiology and Immunology, Medical University of Vienna Kinderspitalgasse 15, A - 1090 Vienna, Austria. Tel.: +43 1 40 160 33150.
*E-mail address:* talin.barisani@meduniwien.ac.at (T. Barisani-Asenbauer).

http://dx.doi.org/10.1016/j.micinf.2015.12.001
1286-4579/

parameters on protection level. In *Chlamydia* research, the guinea pig model using *Chlamydia caviae* for ocular and genital infections is well characterized and accessible [6]. Although a wide range of immunological reagents is lacking for guinea pigs, several research groups were able to characterize important aspects of disease progression and protection in the ocular guinea pig model. Murray reported complete or marked reduction in the intensity of infection upon reinfection [7,8]. Later, Monnickendam et al. described the development of cell-mediated immunity and demonstrated that a trachoma-like disease could be elicited by repeated infections [9,10]; Schachter and co-workers described the local and serum antibody responses as a result of infection and reinfection [11]. More recently, the influence of infectious dose on the ocular infection kinetics and the resulting pathology responses were described in primary infection [12]. In addition, in genital guinea pig model, Rank et al. suggested that the interval between infections is a critical factor in determining whether an animal will develop complete or partial immunity [13,14].

The aim of our study was to further investigate, in the ocular guinea pig model, the interactions between infectious dose, protectivity, pathological and immunologic responses after repeated infections. Here, for the first time, we compared two distinct infectious doses of *C. caviae* and hypothesized that these two infectious doses will differentially shape immune responses and phenotype of disease. The two doses were chosen according to previously established criteria that $1 \times 10^4$ IFU ensures 100% infection in all animals, with a pathology response that can be easily quantified by gross observation [15], and $1 \times 10^6$ IFU which ensures aggravated ocular disease [16].

## 2. Materials and methods

### 2.1. Ethics statement

All experiments were approved by the ''Ethics Committee for the Welfare of Experimental Animals'' at the Institute of Virology, Vaccines and Sera – Torlak and conformed to the Serbian laws and European regulations on animal welfare (Approval No. 011-00-00510/2011-05/2). All animals were handled in strict accordance with good animal practice as defined by the Serbian code of practice (published in Službeni Glasnik No. 41/9) for the care and use of animals for scientific purposes, the Guide for the Care and Use of Laboratory Animals of the Torlak Institute (2133/1, 21. 04. 2011) and a Basel declaration that is committed to the 3R principle (Replace, Reduce, Refine). Animal testing was planned and carried out with extreme care.

### 2.2. Experimental animals

Female, Hartley strain guinea pigs (300–350 g, 6 weeks old) were used in the study. Animals were housed individually in cages with filter tops, given food and water *ad libitum*, and kept on a 12 h light/12 h dark cycle. Guinea pigs were prescreened by in-house optimized ELISA described in detail in 2. 6. section to ensure they were seronegative for *C. caviae*.

### 2.3. Repeated C. caviae conjunctival infections

*C. caviae*, guinea pig inclusion conjunctivitis (GPIC) strain, has been kindly provided by Prof. Roger G. Rank, which has been continually passaged in his laboratory, first in yolk sac and then in tissue culture. Stocks of *C. caviae* were made according to standard methodology in McCoy cells [13] and frozen at −80 °C in sucrose-phosphate-glutamate (SPG) buffer until needed. Guinea pigs were anesthetized intramuscularly with a mixture of ketamine (30 mg/kg) and xylazine (2 mg/kg). First infection was performed on day 0 on anesthetized guinea pigs by instilling 25 μl of SPG buffer containing $1 \times 10^4$ or $1 \times 10^6$ IFUs of *C. caviae* directly into the conjunctival sac with a micropipette. The control group of animals received SPG buffer only. Animals were reinfected after 42 and 126 days. In subsequent infections, guinea pigs received the same dosage as in the 1st infection. Guinea pigs eyes were monitored daily by visual scoring of gross ocular pathology. In addition for each infection, on day 0 (prior to infection) and on days 4, 7, 14 and 21 post-infection, conjunctival swabs and blood were collected to quantify chlamydial IFUs and *C. caviae*-specific IgG levels, respectively. 168 days after the 1st infection (6 weeks after the 3rd infection) a final follow-up was scheduled. The detailed experimental design is presented in Fig. 1.

### 2.4. Pathology scoring

A trained ophthalmologist who was blinded to the experimental groups examined the eyes daily. Conjunctivae were evaluated for erythema, edema, and exudation and the findings were graded as described previously [17,12].

### 2.5. Determination of IFUs from conjunctival swabs

Conjunctival swabs for the isolation and quantification of *C. caviae* were collected from guinea pigs while under ketamine/xylazine anesthesia. Conjunctival swabs were collected with the Copan Universal Transport Medium (UTM-RT) System (Copan, Italy) and frozen at −80 °C until needed. The IFU numbers were determined by culture in McCoy cells, as previously described [18]. In brief, McCoy cell monolayers were infected with swab material using centrifugation, and the infection was stopped after 24 h with 100% methanol fixation. *C. caviae* IFUs were visualized by staining with a fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody against *Chlamydia* lipopolysaccharide (LPS; Clone B410F, Pierce Biotechnology, Rockford, IL, USA). IFUs were counted using a fluorescence microscope (Axio-Observer Zeiss, Vienna, Austria).

### 2.6. Determination of C. caviae-specific IgG in sera

Peripheral blood was taken from the guinea pigs' lateral saphenous veins while the animals were under anesthesia.