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Intragastric primary infection sensitizes to lung reinfection in a *Chlamydia pneumoniae* mouse model

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Summary The most frequently used infection route in *Chlamydia pneumoniae* animal models is intranasal (i.n.), while the intragastric (i.g.) infection route has not been studied previously. The aim of the present study was to examine the course of *Chlamydia pneumoniae* infection in mice infected via the i.g. route and to compare the outcome of i.n. reinfection in these mice to i.n. reinfection of primarily i.n. infected mice. C57BL/6JBom mice were used, and the infection was monitored using chlamydial culture, *C. pneumoniae* PCR and histological examination of several tissues, and antibody measurements. *C. pneumoniae* was able to disseminate from the gastrointestinal tract to other organs, and i.g. inoculation led to an immunological response. In addition, the primary i.g. challenge made mice more susceptible to i.n. reinfection. In conclusion, the results suggest that the nature of the immune response to a previous *C. pneumoniae* infection affects the outcome of reinfection.

Introduction

Chlamydiales are a group of intracellular Gram-negative bacteria that include important human and animal pathogens. Although chlamydial species enter the body through mucosae, they are able to replicate in several other cell types and are thus able to infect several organs and different tissue types. *C. pneumoniae* causes infections of variable severity ranging from mild upper respiratory tract infections to severe pneumonia, and it has also been associated with chronic lung diseases and cardiovascular diseases [1,2].

In chlamydial infections, the factors affecting the disease outcome are complicated and not fully understood. Protection against reinfection by the same *Chlamydia trachomatis* serotype has been demonstrated in an animal

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model [3]. Both in *C. trachomatis* and *C. pneumoniae* infections, protective immunity has been associated with locally produced mucosal antibodies [4,5]. On the other hand, immune responses have also been shown to participate in exaggerated disease-related and pathological changes in affected tissues after repeated infections [6]. There is no effective vaccine against human chlamydial diseases, although several immunogens and several immunization strategies, including oral immunization for *C. trachomatis* and *Chlamydia psittaci*, have been studied. Concerning other infectious diseases, the best-known application of oral vaccination is Sabin's oral polio vaccine. After inoculation, the attenuated poliovirus replicates in the gastrointestinal tract and protects the carrier against severe disease [7]. In experimental chlamydial animal models, too, oral immunization using live chlamydial inoculum has protected against conjunctival, vaginal, or lung infection [8—12]. On the other hand, several studies have reported no protection from oral inoculation [4,13], and one even reported worsening of *C. trachomatis* eye infection in monkeys when UV-inactivated chlamydial inoculum was used [11].

In animal models for *C. pneumoniae* infection, including studies on primary and reinfections as well as pulmonary and cardiovascular disease, the most commonly used inoculation route is intranasal (i.n.) [14,15]. Other routes reported in mice include intracerebral, intravenous, subcutaneous, and intraperitoneal inoculations [16—18]. However, there is no report on the intragastric inoculation route in *C. pneumoniae* infection models, and the present study therefore aimed to examine the course of *C. pneumoniae* infection in mice infected via the intragastric (i.g.) route and to compare the outcome of i.n. reinfection in these mice to that of primarily i.n. infected mice.

Materials and methods

Mice and experimental design

The Animal Care and Use Committee of National Public Health Institute, Helsinki, approved all procedures involving experimental animals in the studies. Eight- to nine-week-old female C57BL/6JBom mice were used ($n = 176$), and they were fed a regular autoclaved chow diet (Altromin, Chr. Petersen A/S, Ringsted, Denmark). Seventy-seven mice were given a primary infectious challenge i.n. during inhaled methoxyflurane anesthesia (Group: i.n.) (Metofane®, Schering-Plough, Kenilworth, NJ, USA), and 77 mice were primarily challenged i.g. without anesthesia into the stomach [14,19]. The i.g. delivery route rather than oral inoculation was chosen after a pilot study where serum *C. pneumoniae* IgG antibodies were measured after oral and i.g. *C. pneumoniae* inoculation. This study showed that, of these two inoculation routes, only i.g. led to *C. pneumoniae* antibody production. A gentle flexible dosing cannula with a Teflon tube and a silicon tip was used for i.g. delivery (B&K Universal AB, Sollentuna, Sweden) (Group: i.g.). The infectious dose with *C. pneumoniae* isolate Kajaani 7 was 1.6×10^6 inclusion-forming units (IFU) in 40 μl of saccharose—phosphate—glutamate (SPG) solution for i.n. inoculations, and the same bacterial load in 200 μl of SPG for i.g. inoculations. Blood and tissue samples were taken from the mice sacrificed with CO_2 at different time points ($n = 7$ mice/group/time point). The mice in the reinfection study were all rechallenged i.n. (Groups: i.n.—i.n. and i.g.—i.n.) at day 42 postinfection (p.i.). The bacterial dose in reinfection was 1.0×10^6 IFU in 40 μl of SPG. Blood and tissue samples were taken similarly as described above.

Chlamydial culture of lung and liver tissue and rectal swab

The right lung and the left lobe of the liver were mechanically homogenized in 2 ml of SPG, and the supernate was separated by centrifugation [14]. The rectal swab was taken with a sterile toothpick and vortexed in 1 ml of SPG. The samples were kept in −70 °C until analysis. Samples were cultured in 24-well plates in HL cells (a human epithelial cell line) in several dilutions. After 72 h of incubation, the cells were methanol-fixed and stained with chlamydia LPS-specific FITC-conjugated monoclonal antibody (pathfinder Chlamydia Confirmation System, Kallestad Diagnostics, Chaska, MN, USA). The results are shown as median IFU/lung ± IQR (25th and 75th percentile).

C. pneumoniae PCR of lung and liver tissue

One lobe of the right lung and half of the median lobe of the liver were taken. DNA was extracted from the tissue samples using a QIAamp tissue kit in accordance with the manufacturer's instructions (Qiagen GmbH). Hot-start PCR was performed using *C. pneumoniae omp1*-specific biotinylated primers (Institute of Biotechnology, Helsinki, Finland) with AmpliTaq polymerase (Promega) and AmpliWax Gem 50 bed (PE Biosystems) in a total reaction volume of 50 μl, and detection of the specific PCR product was done with Eu-labeled SJ1 probes (Institute of Biotechnology) in streptavidin-coated microplates (Wallac) [20]. The mouse samples were run 1—3 times, and a single positive value (exceeding the cut-off value of 50,000 counts per second) was accepted as a positive result.

Histopathology of lungs and other tissues

Inflammatory changes in the lungs (left lung) were determined by histology. A formalin-fixed tissue specimen was embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). The changes in the lungs, as recorded by a pathologist (ES), were graded as no change (histology score = 0), minimal (histology score = 1), slight (histology score = 2), moderate (histology score = 3), or severe (histology score = 4) change, depending on the number of mononuclear cells and the area affected. In the milder forms, the inflammatory cell infiltrates were limited to focal areas or occurred in small, scattered foci, but in the severe cases, large tissue areas were affected. The results are shown as mean ± S.E. of histology scores. Histopathological changes were also searched for in similarly processed liver samples from each mouse (half of the median lobe) as well as in samples of stomach and intestine (gastric and intestinal samples were taken only at day 6 p.i., $n = 2—3$).