Metalloproteinases gene expression in a model of genital *Chlamydia* infection in female Balb/c mice

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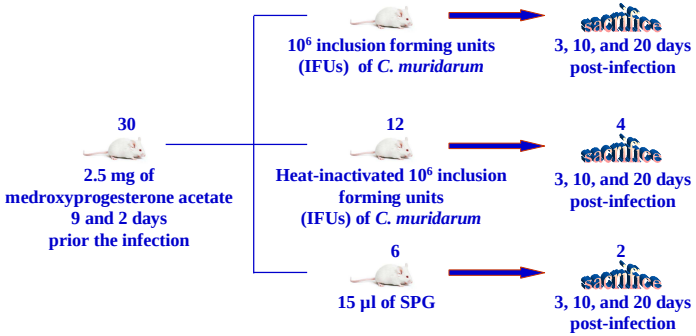
INTRODUCTION AND PURPOSE.

Sexually transmitted *Chlamydia trachomatis* infection is an important public-health concern because of its adverse effects on reproduction. It has become one of the main causes of tubal factor infertility in women due to the ascending infection in the female genital tract and subsequent pelvic inflammatory disease (PID). Although the pathologic consequences of *Chlamydia* genital infection are well-established, the mechanisms leading to tissue damage are not completely understood. Researchers have taken advantage of multiple animal models of chlamydial infection to examine the inflammatory response that occurs in the female genital tract after in vivo inoculation because of difficulties in obtaining information from human tissues. The most widely used genital animal model of *Chlamydia* infection is now the murine infection due to mouse pathogen *C. muridarum*. *C. muridarum* is closely genetically related to *C. trachomatis*, and genital mouse female *C. muridarum* infection is similar to human female infection with *C. trachomatis*: it affords an ascending infection after intravaginal inoculation that leads to salpingitis, endometritis, tubal fibrosis and stenosis, possibly leading to infertility in phenotypically susceptible mice.
In this study we analyzed gene expression of metalloproteinases (MMP-2 and MMP-9) in genital organs obtained from female mice infected by *C. muridarum*.

METHODS.

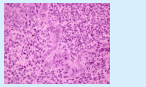
All the experiments were approved by the Ethical Committee of the University of Bologna. Animals used were 30 female Balb/c mice, 6-8 weeks old.



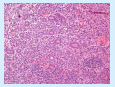
Genital tracts were divided into the cervical-vaginal region, uterine horns, and oviducts.
A portion of each organ was stored in formalin and later processed for histological examinations. The remaining parts were used for RNA extraction, by using Trizol Reagent (Invitrogen), in combination with RNeasy Mini Kit (Qiagen). cDNA was synthesized with 500 ng of total RNA and SuperScript III RT (Invitrogen). Real-time RT-PCR was performed with SYBR Green Fast start kit (Roche Diagnostics). Primers used to assess GAPDH, MMP-2, and MMP-9 levels were from SuperArray (SABiosciences Corporation).

RESULTS.

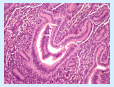
At histological examination no controls showed inflammation. On the contrary, scores of inflammation in all the organs from infected animals peaked at day 10, whereas only a single animal inoculated with inactivated bacteria showed a very mild inflammation at day 10 in its right uterine horn.
At day 10, organs from infected animals showed significantly higher gene expression of MMP-2 and MMP-9 than the respective organs obtained from uninfected mice.



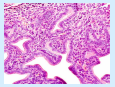
5 days: Endometrial glands attacked by an acute and necrotizing marked infiltrate composed essentially by neutrophils.
The neutrophils are also present in endometrial stroma.



10 days: Many plasma-cells; lymphocites create pseudo-follicles in endometrial stroma. The vascular hyperemia and neovascularization can be easily identified.



20 days: Endometrial glands with mild glandular distorsion after inflammation resolution. Minimum chronic infiltrate in the stroma and only few intraepithelial lymphocites.



10 days: Negative control.

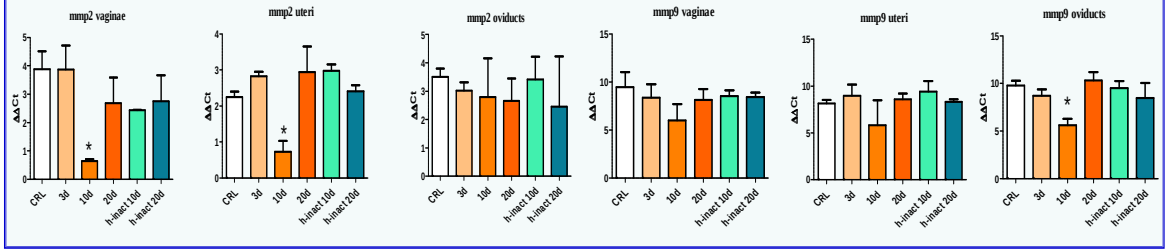
Histological Grading of Uteri	3 days	10 days	20 days
Infected animals	2-3	3-3	1-2
Heat-inactivated chlamydiae treated animals	0-0	0-1	0-0
SPG treated controls	0	0	0

Score grading of uteri obtained from mice inoculated with live chlamydiae, heat-inactivated chlamydiae, or SPG buffer, respectively.

Histological Grading of Oviducts	5 days	10 days	20 days
Infected animals	2-3	3-3	1-2
Heat-inactivated chlamydiae treated animals	0-0	0-0	0-0
SPG treated controls	0	0	0

Score grading of oviducts obtained from mice inoculated with live chlamydiae, heat-inactivated chlamydiae, or SPG buffer, respectively.

Results are shown as expression levels using the ΔΔCt method



Conclusion

Our study showed statistically significant higher MMPs gene expression in infected animals compared to both controls and animals injected with inactivated chlamydiae.
This result confirms the pivotal role of MMPs in the development of tissue damage in genital *Chlamydia* infection.

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