P0120
Poster Session I
Animal models: pathophysiology

METALLOPROTEINASES GENE EXPRESSION IN A MODEL OF GENITAL CHLAMYDIA INFECTION IN FEMALE BALB/C MICE.

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Objectives.

Chlamydia trachomatis genital infections can cause long-term complications in female reproductive tract, even in absence of acute symptoms. The most common complication is pelvic inflammatory disease (PID), that can lead to tubal infertility and extra-uterine pregnancies.

Although the pathologic consequences of *Chlamydia* genital infection are well-established, the mechanisms leading to tissue damage are not completely understood. 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*, closely mimicking human infections in the mouse model.

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. All the animals received medroxyprogesterone acetate 9 and 2 days prior the infection.

Twelve mice were infected by placing 15 µl of sucrose-phosphate-glutamic acid (SPG) buffer containing 10^6 inclusion forming units (IFUs) of *C. muridarum* into the vaginal vault. Twelve animals were treated with 15 µl of SPG containing heat-inactivated 10^6 IFUs of *C. muridarum*. As controls of inflammation, 6 animals were challenged with 15 µl of SPG.

At 3, 10, and 20 days post-infection 4 infected animals, 4 animals inoculated with heat-inactivated bacteria and 2 controls were sacrificed.

Genital tracts were divided into the cervical-vaginal region, uterine horns, and oviducts.

A part of uterine horns, oviducts and vagina were stored in formalin and later processed for histological examinations. The remaining parts of the organs 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 in Real-time RT-PCR 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 non-infected mice.

Conclusions.

Our study showed statistically significant higher MMPs gene expression in infected animals compared to both controls and animals injected with inactivated chlamydiae.

These results confirm the pivotal role of MMPs in the development of tissue damage observed during *Chlamydia* genital infection.