Chlamydia trachomatis and Chlamydophila abortus induce the expression of secretory leukocyte protease inhibitor in cells of the human female reproductive tract

Citation for published version:

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Microbiology and Immunology

Publisher Rights Statement:
Copyright 2008 The Societies and Blackwell Publishing Asia Pty Ltd

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and/or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Chlamydia trachomatis and Chlamydophila abortus induce the expression of secretory leukocyte protease inhibitor in cells of the human female reproductive tract

Nick Wheelhouse¹, Sean Wattegedera¹, Diana Fleming², Paul Fitch³, Rodney Kelly² and Gary Entrican¹

¹Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, EH26 0PZ ²MRC Human Reproductive Sciences Unit and ³MRC/University of Edinburgh Centre for Inflammation Research, Little France Crescent, Edinburgh, EH16 4TJ, UK

ABSTRACT

C. trachomatis and C. abortus are related Gram-negative intracellular bacteria that cause reproductive failure due to infertility (C. trachomatis) or abortion (C. abortus). These organisms target epithelial cells in the reproductive tract and/or placenta, but the innate immune mechanisms that lead to protection or pathology and disease are poorly understood. SLPI is an innate immune molecule which protects mucosal surfaces from infection and injury. C. trachomatis and C. abortus were found to induce SLPI mRNA and peptide expression in HeLa (cervical epithelium) and JEG-3 cells (trophoblast) respectively. Both cell lines constitutively expressed SLPI and, although infection enhanced this expression, killed organisms did not. These data demonstrate that Chlamydia/Chlamydophila grow in cells that express SLPI, suggesting that SLPI does not exert antimicrobial effects against these organisms. However, SLPI has multiple functions, and we speculate that it may play a role in controlling tissue inflammation and pathology.

Key words Chlamydia, innate immunity, secretory leukocyte protease inhibitor

C. trachomatis and C. abortus, both obligate intracellular bacterial pathogens belonging to the genus Chlamydiae, are associated with reproductive failure (1). C. trachomatis infects epithelial cells of the lower and upper reproductive tracts and has a strong association with infertility and ectopic pregnancy. These outcomes have been linked to inflammatory host immune responses (2). C. abortus infects placental trophoblast and causes abortion in sheep and humans. Again, this response is linked to local immunopathology (3). Both pathogens can induce chronic infections. The early events following primary (acute) infection are likely to be very important in determining progression to chronic infection. Given that acute chlamydial infections are characterized by intense inflammation and mucosal infiltration of immune cells including neutrophils (4), and that Chlamydiae are susceptible to natural antimicrobials (5, 6), it was hypothesized that innate immunomodulatory molecules may play a role in chlamydial infection of epithelial cells in the reproductive tract.

SLPI is a highly conserved proteinase inhibitor that is constitutively expressed by epithelial cells, mast cells, neutrophils and macrophages. SLPI is thought to protect mucosal surfaces from damage during inflammation (7) acting as a neutrophil elastase inhibitor (8) and controlling...
inflammation by down-regulating TNF-α production in LPS-activated macrophages (9). SLPI also exhibits antimi-
crobial properties against both Gram-negative and Gram-
positive bacteria (7).

Expression of SLPI at mucosal sites such as the res-
piratory and gastrointestinal tracts can be modulated,
both positively and negatively, by pathogens. For example,
Staphylococcus aureus (10) and Helicobacter pylori (11)
can inhibit SLPI expression whereas HIV-1 (12) and M.
tuberculosis (13) can augment SLPI expression. SLPI is
expressed throughout the female reproductive tract (14)
and in human trophoblasts (15). It is an antibacterial con-
stituent of vaginal fluid (16) and cervical mucus (17). Fur-
thermore, the bactericidal activity of uterine cell washes
in culture can, at least partially, be inhibited by antibodies
that neutralize SLPI (18). Thus, it is of interest to in-
vestigate modulation of SLPI expression by pathogens of
the reproductive tract with a view to understanding the
complex host-pathogen interactions that can lead to pro-
tection or immunopathology. To address our hypothesis,
SLPI expression in HeLa 229 (derived from human cervical
epithelium) and JEG-3 cells (derived from human placen-
tal chorial epithelium) infected with C. trachomatis and
C. abortus respectively, was investigated.

HeLa cells were grown to sub-confluence in IMDM, (In-
vitrogen, Paisley, UK) supplemented with 2% FBS (PAA
laboratories, Teddington, UK) in 24 well plates (Corning
Costar, High Wycombe, UK). Cells (2 × 106 cells in 1ml)
were infected with C. trachomatis (Serovar E) at an esti-
mated MOI of 0.1. The MOI was derived by evaluating the
number of inclusion-forming units of the parent stock of
C. trachomatis EB titrated on HeP-2 cells. Infected cells
were fixed 48 hr after infection and inclusions stained us-
ing the 13/4 anti-chlamydial LPS monoclonal antibody
followed by a rabbit anti-mouse FITC conjugated anti-
body. Uninfected cells served as controls. The cells were
cultured for 4, 8, 24 or 48 hr, after which time the su-
pernatants were harvested and stored at −20°C until further
analysis for the presence of SLPI peptide by ELISA (Hycult
Biotech, Uden, the Netherlands). At harvest, the cells were
washed with cold PBS and RNA isolated using the Qiagen
RN easy mini kit (Qiagen, Crawley, UK) for analysis of
the presence of mRNA encoding SLPI by quantitative real-
time RT-PCR. All reagents and equipment used for cDNA
synthesis and real-time RT-PCR analysis were from Ap-
plied Biosystems (Applied Biosystems, Warrington, UK)
unless otherwise stated. One μg of total RNA was reverse
transcribed using the Taqman Reverse transcription kit.
For the real-time PCR, 50 ng of cDNA was amplified using
the forward primer 5′-TGACACCTTGTGGACATAAATG-
3′, the reverse primer 5′-CCCAGGCTTCCTCCTTGT-3′
and the probe 5′-GGATCCGTGTGGACACCCCAACC-3′
using the Taqman Master Mix, on an ABI 7000 Prism real-
time thermo cycler. 18S rRNA was amplified in a duplex
reaction using a validated primer: probe set as internal
control and to allow quantitation relative to baseline un-
infected control cells. Experiments were repeated at least
three times. Due to evidence of increasing variability with
increasing means, results were log10 transformed prior to
statistical analysis by two-way ANOVA which was used to
generate 95% confidence intervals.

Relative to uninfected controls, SLPI mRNA expression
was down regulated 4 hr after C. trachomatis infection
(P < 0.05; Fig. 1a). However, 24 and 48 hr after infection
there was a greater than 2.5 fold increase relative to un-
infected cells (Fig. 1a). The increase in mRNA expression
following infection was reflected in an increase in SLPI
peptide in the culture supernates after 48 hr (P < 0.05;
Fig. 1b). Measurement of SLPI peptide in the culture
supernates revealed constitutive expression in uninfected
cells. Despite the constitutive and induced expression, C.
trachomatis grows well in HeLa cells suggesting that SLPI
does not exert strong anti-chlamydial effects (19).

Since HeLa is a cell line widely-used to study C.
trachomatis biology, the question of whether the phe-
nomenon of SLPI induction is unique to that particu-
lar host-pathogen interaction is of interest. The closely-
related organism C. abortus was therefore studied in a dif-
f erent natural target cell (trophoblast). Experiments were
conducted using the same protocols as described for C.
trachomatis infection of HeLa cells with minor modifi-
cations as outlined below. Briefly, JEG-3 chorionic tro-
phoblast cells were grown to sub-confluence in 24 well
plates and infected with C. abortus at an estimated MOI
of 0.1, 1 or 10. The C. abortus stock (S26/3) was grown
in HeP2 cells and titrated as described above for C. tra-
chomatis with the exception that cells were fixed 72 hr
after infection. A lysate of uninfected HeP2 cells and UV-
killed C. abortus EB were used as further controls. The
uninfected cell lysate was used at dilutions that matched
those of the infected cells. EB were exposed to UV at
254 nm, 20 mJ and inactivation was confirmed by the fail-
ure of C. abortus to grow in HEp-2 cells as determined
using the same staining protocol as that used for the titra-
tion. Supernates and cell lysates were harvested 24, 48
and 72 hr after infection and analyzed for SLPI protein
and mRNA as described above. The results are shown in
Figure 2.

A dose-dependent and time-dependent increase in SLPI
mRNA expression was observed at MOI of 1 and 10 at 48 hr
(2 and 7-fold respectively; P < 0.05 compared to uninf-
ected cells) and 72 hr (4 and 8-fold respectively; P < 0.05
compared to uninfected cells, Fig. 2a). As for HeLa cells,
JEG-3 trophoblasts were found to constitutively release
SLPI peptide into the culture supernates (Fig. 2b). Again,
a significant increase was observed following infection with
Induction of SLPI by Chlamydia

Fig. 1. SLPI expression in HeLa cells infected with C. trachomatis serovar E (MOI 0.1). (a) SLPI mRNA expression. Each value represents the fold change compared to the uninfected/untreated control cells at that time point. 4 hr (lined bar), 8 hr (white bar), 24 hr (black bar), 48 hr (grey bar). (b) SLPI protein secretion into the culture supernatant. 4 hour (lined bar), 8 hour (white bar), 24 hour (black bar), 48 hour (grey bar). Error bars represent the standard error of the means of four independent experiments. *P < 0.05 increase relative to medium alone values at the specific time point.

Fig. 2. SLPI expression in JEG-3 cells, infected with C. abortus at MOI 0.1, 1, 10, UV treated organisms or exposed to a HEp2 cell lysate prepared using the same method used for the propagation of C. abortus. (a) SLPI mRNA expression. Each value represents the fold change compared to the control uninfected sample (medium alone) at that time point. (b) SLPI protein secretion into the culture supernatant. 24 hr (lined bar), 48 hr (white bar), 72 hr (black bar). Experiments were carried out on at least three separate occasions. Error bars represent the standard error of the means. *P < 0.05 different from medium control values at the specific time point.

C. abortus at MOI of 1 (P < 0.05) and MOI of 10 at 72 hr (P < 0.05) compared to uninfected controls (Fig. 2b).

However, there was no increase in SLPI mRNA expression or peptide when JEG-3 cells were exposed to UV-C-irradiated (killed) C. abortus or treated with cell lysates (Fig. 2a, b). The failure of killed organisms to induce SLPI expression by trophoblast indicates that chlamydial invasion and/or multiplication is an essential element in this process. It also suggests that the sensory mechanism that detects the organism, and subsequently promotes SLPI expression, is likely to be an intracellular receptor as opposed to a cell-surface exposed pattern recognition receptor.

These data demonstrate that C. trachomatis and C. abortus infection stimulate SLPI expression by cells in the female reproductive tract. The requirement for invasion and/or multiplication is interesting since both live and heat-killed M. tuberculosis stimulate the expression of SLPI mRNA in macrophages (13). The production of antimicrobial molecules following chlamydial infection is not without precedent. Defensins have been found in patients with C. trachomatis-induced urethritis (20). However, given that both C. trachomatis and C. abortus grow very well in cells that constitutively express SLPI, and moreover induce further SLPI expression, we deduce that SLPI does not exert potent antimicrobial effects on these organisms. This raises the question as to the function of SLPI during chlamydial infection. We speculate that its role is to control inflammation in a manner that
supports chlamydial survival in vivo, possibly via mechanisms that interfere with neutrophil clearance of infection. This remains to be addressed.

ACKNOWLEDGMENTS

We thank Jill Sales (BioSS) for expert statistical advice and analyses, and Ian Clarke and Martin Holland for critical reviews. We also thank Plotkowski M.C., Puchelle E. (2004) Dynamic interaction between airway epithelial cells and Staphylococcus aureus. Am J Physiol Lung Cell Mol Physiol 287: L543–L551.

REFERENCES


© 2008 The Societies and Blackwell Publishing Asia Pty Ltd