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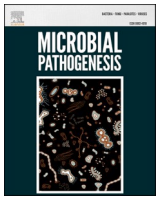

Interferon-ε as potential inhibitor of *Chlamydia trachomatis* infection

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ABSTRACT

Chlamydia trachomatis, the main cause of bacterial sexually transmitted diseases, is responsible for severe reproductive sequelae. Amongst all the cytokines involved in host immunity towards this pathogen, IFN-ε has recently acquired importance for its potential contribution to the female reproductive tract innate defenses. Herein, our study aimed to explore, for the first time, the activity of IFN-ε toward *C. trachomatis* in an *in vitro* infection model, by testing its effects on the different phases of chlamydial developmental cycle, as well as on the ultrastructural characteristics of chlamydial inclusions, via transmission electron microscopy. Main result is the capability of IFN-ε to alter *C. trachomatis* growth, as suggested by reduced infectious progenies, as well as a patchy distribution of bacteria and altered morphology of reticulate bodies within inclusions. In conclusion, our results suggest that IFN-ε could play a role in the innate and adaptive immune defenses against *C. trachomatis*; in the future, it will be needed to investigate its activity on an infection model more closely resembling the physiological environment of the female genital tract.

1. Introduction

Chlamydia trachomatis is the leading cause of bacterial sexually transmitted diseases, with more than 130 million new cases worldwide, according to the most recent World Health Organization (WHO) estimates [1,2]. *C. trachomatis* genital infection is mainly responsible for urethritis and cervicitis in women, and urethritis in men, although more than 90% of all cases in women and 50% in men are asymptomatic, and, hence, untreated, leading to severe reproductive sequelae such as pelvic inflammatory disease (PID) and ectopic pregnancy in women, prostatitis and orchitis in men, as well as infertility in both genders [3–6].

C. trachomatis is an obligate intracellular bacterium with a unique biphasic developmental cycle, occurring into a phagocytic vacuole termed inclusion and alternating between the extracellular infectious elementary body (EB), responsible for adhesion and invasion into the host cell, and the intracellular replicative reticulate body (RB), responsible for multiplication within the inclusion [7,8]. Under stressful growth conditions, such as, for example, interferon (IFN)γ exposure, *C. trachomatis* has been demonstrated to alter its biological state to generate viable but non-cultivable forms called persistent forms [9–11], able to evade the host immune response and more difficult to eradicate by antibiotics [12–15].

The host innate immune response in the reproductive tract represents the first line of defense against bacterial infections and, amongst all of the inflammatory cytokines involved, type-I and II Interferons (IFN–I/-II) play an important role in the defense against *C. trachomatis* [16,17]. Generally, following *C. trachomatis* infection, target cells secrete IFN-I, primarily the most extensively studied IFNα subtypes and IFNβ, that promotes the recruitment of T-lymphocytes and other inflammatory cells [18,19]. Their increase in response to *C. trachomatis* genital infection leads to the synthesis of IFNγ, an IFN-II, known as the predominant response for the clearance of *C. trachomatis* [20,21], as evidenced by several *in vivo* and *in vitro* studies [22–27].

Amongst the other less common IFNs belonging to the IFN-I family,

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IFN-ε has acquired importance, in recent years, as it may also contribute to the first line of defense against *C. trachomatis*. Interestingly, IFN-ε-deficient mice have been demonstrated to possess increased susceptibility to vaginal infections by sexually transmitted pathogens, like *C. muridarum* [28]. Unlike IFN-α and IFN-β, IFN-ε is constitutively expressed in various mucosal tissues [29–31]. Specifically, it is highly expressed in the epithelial cells of the female reproductive tract, especially in the uterus, cervix, vagina, and ovary [32,33], it is also correlated to the estrous cycle, since estrogen is able to induce its synthesis, decreases during pregnancy, and persists throughout the course of an infection [30].

Therefore, this raises the interesting question of whether IFN-ε may possess anti-microbial activity towards *C. trachomatis*, potentially leading to the clearance of the genital infection. On this regard, it has been investigated, for the first time, its effects in the different phases of chlamydial developmental cycle.

2. Materials and methods

2.1. Reagents and cell lines

Escherichia coli-derived human IFN-ε protein Leu22-Arg208 (Cys175Ser), with a predicted molecular mass of 23 kDa (kDa) and an N-terminal Met and a C-terminal 6-His tag (R&D Systems, Inc. a Bio-Techne Brand, Minnesota, USA) has been used in this study.

McCoy cell line (ECACC, Public Health England, catalogue number 90010305, Porton Down, Salisbury, UK) were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS) at 37 °C in a humidified atmosphere with 5% CO_2. Upon confluency (>85%), cells were passaged with brief trypsinization.

2.2. Propagation and titration of C. trachomatis

C. trachomatis serovar D strain UW3 (VR-855, ATCC, Manassa, VA, USA) was propagated in McCoy cells, as previously described [7]. Briefly, confluent McCoy cell monolayers were infected with chlamydial EBs by centrifugation at 754×*g* for 30 min, and then harvested by scraping after 44 h post infection. The resulting suspension was, then, vortexed with sterile glass beads for 2 min and the supernatant, containing chlamydial EBs, was added to equal volume of 4 × Sucrose Phosphate (4SP) buffer, and stored at −80 °C.

For *C. trachomatis* titration, McCoy cell monolayers were infected with 10-fold serial dilutions of bacterial stock, incubated for 48 h at 37 °C, fixed with methanol and stained with isothiocyanate-conjugated monoclonal antibody anti-*C. trachomatis* LPS (Merifluor® Chlamydia, Meridian Bioscience Inc., Cincinnati, OH, USA), as previously described [34]. The total number of *C. trachomatis* Inclusion Forming Units (IFUs) was enumerated by counting all microscope fields using a fluorescence microscope (400 × magnification).

2.3. Interferon-ε cytotoxicity

Confluent McCoy cell monolayers, grown on 96 wells cell culture trays, were incubated with increasing concentrations of IFN-ε (10 ng/mL, 50 ng/mL, 100 ng/mL and 150 ng/mL) in DMEM supplemented with 10% FBS at 37 °C in humidified atmosphere with 5% CO_2. After 24 h, the number of viable cells was determined via MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay, as previously described [35,36].

2.4. Effects of IFN-ε on the different phases of C. trachomatis developmental cycle

IFN-ε was tested at different concentrations (25 ng/mL, 50 ng/mL, and 100 ng/mL) for its anti-chlamydial activity on the different phases of *C. trachomatis* developmental cycle, specifically the chlamydial EBs pre-treatment with IFN-ε, McCoy cell monolayers pre-incubation with IFN-ε followed by *C. trachomatis* EBs infection, and treatment of McCoy cell monolayers infected with *C. trachomatis* EBs.

2.4.1. Pre-treatment of C. trachomatis EBs with IFN-ε

C. trachomatis (5 000 EB/mL, corresponding to a multiplicity of infection, MOI, of 0.05), were pre-incubated in DMEM supplemented with 10% FBS, in the absence or presence of IFN-ε (25 ng/mL, 50 ng/mL and 100 ng/mL), for 2 h at 37 °C in humidified atmosphere with 5% CO^2. Subsequently, *C. trachomatis* EBs suspension was used to infect McCoy cell monolayers grown as above described. Subsequently, the cells were washed with DPBS to remove non-internalized *C. trachomatis* EBs and newly incubated in fresh culture medium, consisting of DMEM supplemented with 10% FBS. After 44 h post infection at 37 °C and 5% CO_2, the total number of *C. trachomatis* IFU was determined by immunofluorescence assay (IFA), as above described.

2.4.2. Pre-incubation of McCoy cell monolayers with IFN-ε

McCoy cell monolayers, grown on glass coverslips in 24 wells cell culture trays, were pre-incubated in DMEM supplemented with 10% FBS, in the absence or presence of IFN-ε (25 ng/mL, 50 ng/mL and 100 ng/mL). After 24 h of incubation at 37 °C and 5% CO_2, IFN-ε was removed by washing the cells 3 times with DPBS, and, subsequently, pre-incubated and control McCoy cell monolayers were infected with *C. trachomatis* at a MOI of 0.05 as above described. After 44 h post infection at 37 °C and 5% CO_2, the total number of *C. trachomatis* IFU was determined by IFA.

2.4.3. Treatment of C. trachomatis-infected McCoy cell monolayers with IFN-ε

McCoy cell monolayers, grown on glass coverslips in 24 wells cell culture trays, were infected with *C. trachomatis* at a MOI of 0.05, as above described. Subsequently, the cells were washed with DPBS to remove non-internalized *C. trachomatis* EBs and fresh medium, with or without IFN-ε (25 ng/mL, 50 ng/mL and 100 ng/mL), was added to the infected cells. After 44 h post infection at 37 °C and 5% CO_2, the total number of *C. trachomatis* IFU was determined by IFA.

2.5. Assessment of C. trachomatis infectious progeny after IFN-ε treatment

McCoy cell monolayers, grown on glass coverslips in 24 wells cell culture tray, were infected with *C. trachomatis* EBs at a MOI of 0.05 and then treated with IFN-ε at the highest concentration tested (100 ng/mL) as described above. Subsequently, infectivity yield was determined by the development of inclusions after passage to fresh McCoy cell monolayers. Briefly, *C. trachomatis*-infected McCoy cell monolayers, with or without IFN-ε, were disrupted by vortexing for 2 min with sterile glass beads and repassed onto fresh McCoy cell monolayers grown on glass coverslips in 24 wells cell culture tray. After 44 h of incubation at 37 °C and 5% CO_2, the total number of *C. trachomatis* IFU was determined by IFA.

2.6. Transmission electron microscopy of C. trachomatis inclusions following IFN-ε treatment

Confluent McCoy cell monolayers, grown in 25cm^2 cell culture flasks, were infected with *C. trachomatis* at a MOI of 0.05 and then, treated with IFN-ε (100 ng/mL). After 44 h of incubation at 37 °C and 5% CO_2, McCoy cells were fixed in 2% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M sodium cacodylate buffer containing 3 mM $CaCl_2$ and 0.1 M sucrose (pH 7.4), at room temperature for 30 min, and stored at 4 °C. After overnight fixed cells were scraped, washed in 0.15 M sodium cacodylate buffer containing 3 mM $CaCl_2$ (pH 7.4) and centrifuged. The pellets were resuspended and postfixed in 2% osmium tetroxide in 0.07 M sodium cacodylate buffer containing 1.5 mM $CaCl_2$

(pH 7.4) at 4 °C for 2 h, dehydrated through graded ethanol concentrations and embedded in Epon 812 resin (Electron Microscopy Science, Fort Washington, PA). Ultrathin sections, obtained with a Leica UC6 ultramicrotome (Leica Microsystems, Wetzlar, Germania), were contrasted with UranyLess EM Stain (Electron Microscopy Sciences, Hatfield, PA) and lead citrate and examined with a Philips 208S transmission electron microscope (FEI Company, Eindhoven, The Netherlands).

2.7. Statistical analysis

All values are expressed as means ± standard deviation (SD) of two to four replicates from at least two independent *in vitro* experiments. Comparisons of means were performed by using a two-tailed Student t-test for independent samples. The single or multiple inference significance level was set to 5%. All statistical calculations and graphs were performed in the software Excel (Microsoft, USA, version 2302, build 16130.20332).

3. Results

3.1. IFN-ε cytotoxicity

As shown in Fig. 1, no statistically significant decrease in cell viability was observed for IFN-ε treatment of McCoy cell monolayers, at the concentrations 150 ng/mL, 100 ng/mL, 50 ng/mL and 5 ng/mL.

3.2. Anti-chlamydial properties of IFN-ε

First, the anti-chlamydial activities of IFN-ε were investigated toward the extracellular infectious bodies of *C. trachomatis*, pre-treated with IFN-ε at the following concentrations: 100 ng/mL, 50 ng/mL and 25 ng/mL. The IFN-ε did not show the ability to interfere with the infectivity of *C. trachomatis* EBs, as evidenced by no statistically significant reduction in the total number of chlamydial IFUs as compared to untreated chlamydial EBs, even at the highest concentration (100 ng/mL, Fig. 2A).

Then, the potential anti-chlamydial effects of IFN-ε (100 ng/mL, 50 ng/mL and 25 ng/mL) were evaluated after incubating McCoy cells with IFN-ε, followed by *C. trachomatis* infection (pre-incubation phase), or after treating with IFN-ε of *C. trachomatis* infected cells (treatment phase). As shown in Fig. 2B, no statistically significant decrease in the total number of *C. trachomatis* IFUs was observed in the pre-incubation phase with all IFN-ε concentrations tested. A slight reduction in the number of *C. trachomatis* IFUs could be observed in the treatment phase, although it did not reach statistical significance. No statistically significant effect on chlamydial inclusion size was observed in all treatment conditions.

3.3. IFN-ε reduces C. trachomatis infectious progenies

To investigate whether the IFN-ε treatment of *C. trachomatis* could impair the production of infectious EBs, the number of chlamydial infectious progenies was assessed following IFN-ε treatment at 100 ng/mL. As shown in Fig. 3, a statistically significant decrease in the number of infectious progenies was observed in cell monolayers infected with *C. trachomatis* and then treated with IFN-ε, resulting in a total number of chlamydial IFUs approximately 250 times lower than that in untreated *C. trachomatis* infected cells ($p = 0.02$), with a progeny number per EB of 129.1 in untreated *C. trachomatis*, as compared to 0.92 in treated *C. trachomatis* ($p = 0.02$).

To confirm that the decrease in infectious progeny number could be due to an impaired RB differentiation, transmission electron microscopy analysis of chlamydia infected cell was performed. As evidenced in Fig. 4, cells infected with *C. trachomatis* and treated with IFN-ε showed visible difference in distribution or morphology of chlamydial RBs and EBs within inclusions, as evidenced by few enlarged and morphologically aberrant bodies and reduced bacterial density, as compared to untreated cells infected with *C. trachomatis* (Fig. 4).

4. Discussion

The main result of our study is the demonstration of IFN-ε capability to alter *C. trachomatis* growth, as shown by a reduced production of infectious EBs and altered morphology of inclusions. Indeed, a decrease of more than 200 times in the infectious progeny number were observed in the presence of IFN-ε, alongside few enlarged and morphologically aberrant bodies, as well as heterogeneous distribution of chlamydial RBs and EBs within inclusions.

The compelling anti-chlamydial properties of IFN-ε observed in our study suggest that this cytokine might play a role in the clearance of *C. trachomatis* genital infection. This protective effect has also been observed against other sexually transmitted pathogens, like Herpes Simplex Virus-2 (HSV-2) and *C. muridarum* [28], as well as HIV and Zika virus infection [37–41]. Also, the observed decrease in the number of infectious EBs after treatment with IFN-ε might halt the dissemination of *C. trachomatis* in the host, leading to a lower severity of disease and risk for chronic reproductive complications. Our findings are also supported by an *in vivo* study, showing increased susceptibility to *C. muridarum*, typically used as murine model for *C. trachomatis* genital infection.

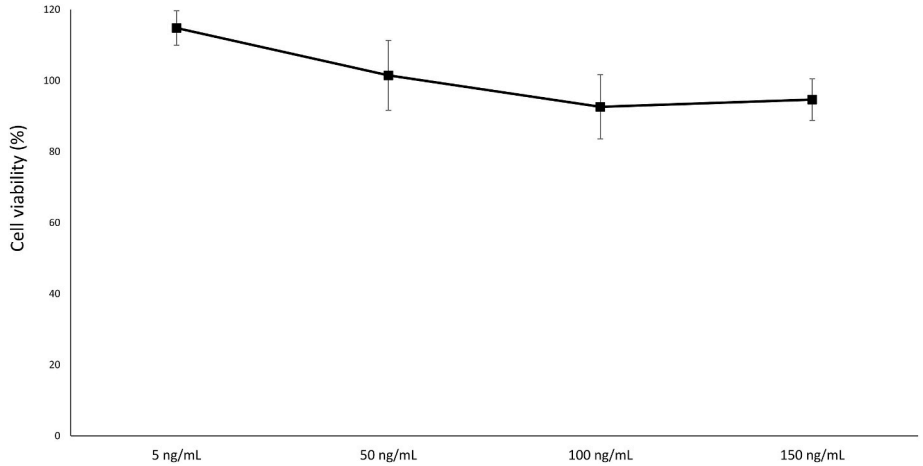

Fig. 1. IFN-ε cytotoxicity. McCoy cells were overlayed with solutions containing decreasing concentrations of IFN-ε, from 150 ng/mL to 5 ng/mL, and then incubated for 24 h at 37 °C and 5% CO_2.

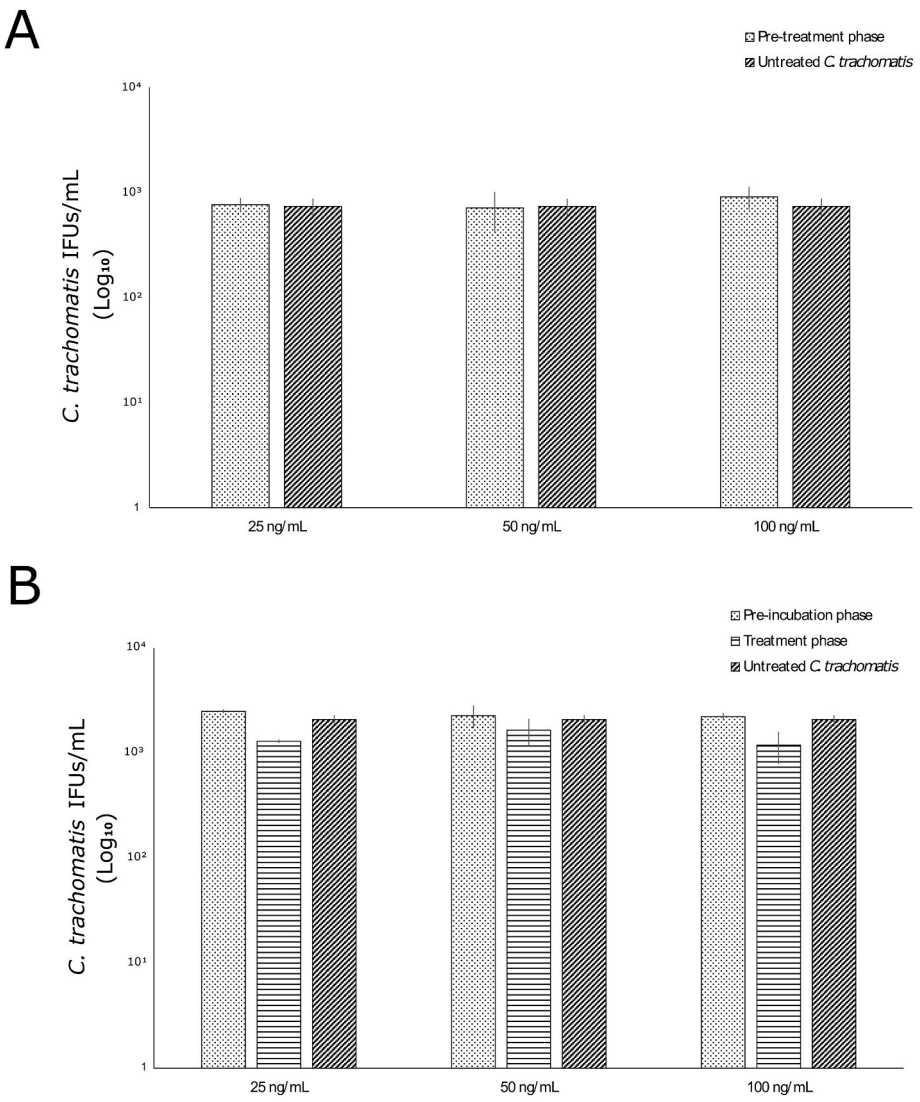

Fig. 2. IFN-ε activity against *C. trachomatis*. Pre-treatment of *C. trachomatis* EBs (A); pre-incubation and treatment phases (B). In the-pre-incubation phase, McCoy cells monolayers were first exposed to different concentrations of IFN-ε followed by *C. trachomatis* infection at a MOI of 0.05 (primary infection); in the treatment phase, McCoy cell monolayers were first infected with *C. trachomatis* at a MOI of 0.05 and then treated with different concentrations of IFN-ε. The total number of IFUs was enumerated after 44 h post infections via fluorescence microscopy.

Indeed, higher bacterial recovery was observed in the reproductive tract of IFN-ε-deficient mice throughout the course of the infection, accompanied by a more severe course of the diseases, as compared to wild type mice [28].

In our study, no anti-microbial effects of IFN-ε were reported against the initial phases of chlamydial growth cycle, as also described in other bacterial and viral pathogens, like *Mycobacterium tuberculosis,* Simian immunodeficiency virus, Theiler's murine encephalomyelitis virus, and Mengovirus infections [29,42]. On this regard, the plethora of different effects of IFN-ε towards *C. trachomatis* and other pathogens might be explained by a specific activity of IFN-ε in relation to the type of microorganism and highlights the complexity of the molecular and cellular events mediated by this cytokine.

Overall, our results suggest that IFN-ε could be considered an important cytokine of the innate and adaptive immune defenses against *C. trachomatis*, similarly to IFN-γ, which is well known to play a key role in the clearance and protection against this pathogen [13,27]. IFN-γ and IFN-ε belong to two different IFN types an act via different mechanisms; IFN-γ reduces tryptophan availability through the upregulation of the enzyme indoleamine 2,3-dioxygenase (IDO), thus inhibiting chlamydia intracellular replication [43], whereas IFN-ε mostly activates JAK/STAT signalling and regulate the expression of IFN-regulated genes (IRGs), involved in antiviral activities and cell cycle regulation [44].

The main strength of our paper lies in the set-up of a *C. trachomatis* infection model using a McCoy fibroblast cell line that is known to not constitutively synthesize IFN-ε [30], allowing us to better investigate the potential anti-chlamydial effects of this cytokine [41]. Moreover, the range of concentrations (25–100 ng/mL) of IFN-ε assayed correspond to those of well characterized IFN-I, such as IFNα and β [45].

In the future, in order to deepen our knowledge on the interplay of IFN-ε and *C. trachomatis*, it will be needed to investigate its activity on an infection model more closely resembling the physiological environment of the female genital tract, via the adoption, for example, of *in vitro* models using cervical epithelial primary cells, or more complex tissue models including organoids.

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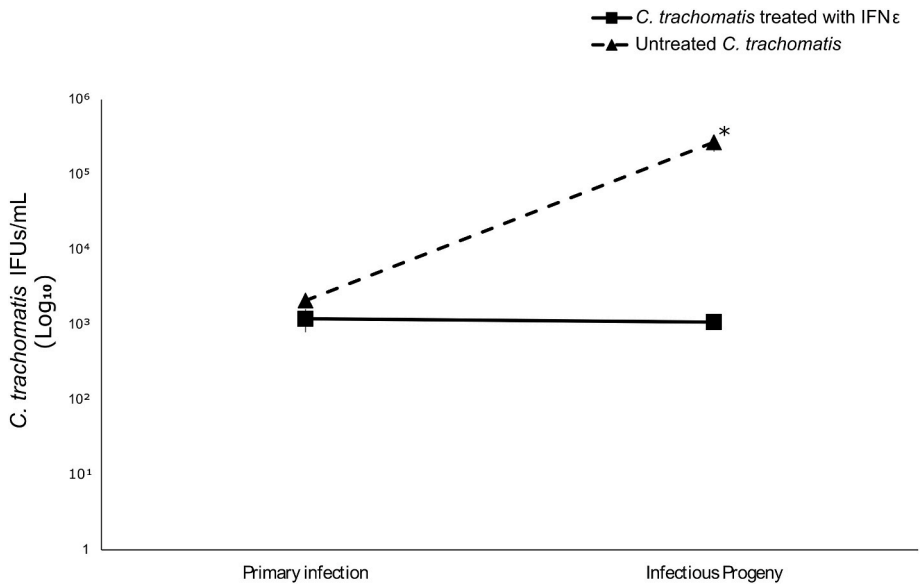

Fig. 3. IFN-ε reduces the number of *C. trachomatis* infectious progenies. Chlamydia infected cell monolayers treated with IFN-ε, alongside untreated chlamydial infected cells as control (primary infection), were harvested and used to infect fresh cell monolayers (infectious progeny). The total number of chlamydial IFUs was enumerated at 44 h post infection via fluorescence microscopy. *, $p < 0.05$.

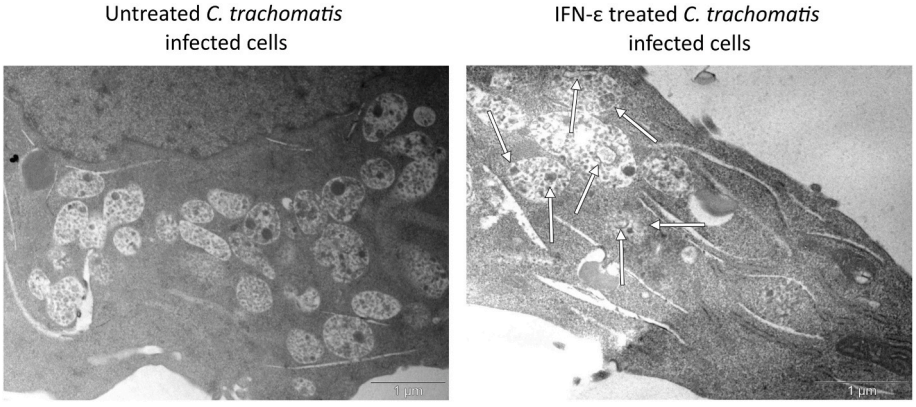

Fig. 4. Transmission electron micrographs of *C. trachomatis* infected cells in the presence of IFN-ε. Chlamydia infected cell monolayers were treated with IFN-ε (100 ng/mL) and, at 44 h post infection, examined via transmission electron microscopy, at 1 μm magnification level. White arrows show aberrant bodies.

CRediT authorship contribution statement

Simone Filardo: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marisa Di Pietro:** Writing – original draft, Visualization, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giuseppina Bozzuto:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation. **Matteo Fracella:** Writing – review & editing, Methodology, Formal analysis. **Camilla Bitossi:** Writing – review & editing, Methodology, Formal analysis. **Agnese Molinari:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Carolina Scagnolari:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization. **Guido Antonelli:** Writing – review & editing, Validation, Supervision, Resources. **Rosa Sessa:** Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rosa Sessa reports article publishing charges was provided by University of Rome "Sapienza". If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] J. Rowley, S. Vander Hoorn, E. Korenromp, N. Low, M. Unemo, L.J. Abu-Raddad, R.M. Chico, A. Smolak, L. Newman, S. Gottlieb, S.S. Thwin, N. Broutet, M. M. Taylor, Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, Bull. World Health Organ. 97 (2019), https://doi.org/10.2471/BLT.18.228486, 2016.

[2] Global progress report on HIV, viral hepatitis and sexually transmitted infections, in: 2021. Accountability for the Global Health Sector Strategies 2016–2021: Actions for Impact, World Health Organization, Geneva, 2021.

[3] C.M. O'Connell, M.E. Ferone, Chlamydia trachomatis genital infections, Microb. Cell 3 (2016), https://doi.org/10.15698/mic2016.09.525.

[4] R. Rodrigues, P. Vieira-Baptista, C. Catalão, M.J. Borrego, C. Sousa, N. Vale, Chlamydial and gonococcal genital infections: a narrative review, J. Personalized Med. 13 (2023) 1170, https://doi.org/10.3390/jpm13071170.

[5] Y. Zuo, T.-T. Jiang, Y. Teng, Y. Han, Y.-P. Yin, X.-S. Chen, Associations of Chlamydia trachomatis serology with fertility-related and pregnancy adverse outcomes in women: a systematic review and meta-analysis of observational studies, EBioMedicine 94 (2023), 104696, https://doi.org/10.1016/j.ebiom.2023.104696.

[6] R. Henkel, Long-term consequences of sexually transmitted infections on men's sexual function: a systematic review, Arab. J. Urol. 19 (2021) 411–418, https://doi.org/10.1080/2090598X.2021.1942414.

[7] R.J. Skilton, Y. Wang, C. O'Neill, S. Filardo, P. Marsh, A. Bénard, N.R. Thomson, K. H. Ramsey, I.N. Clarke, The Chlamydia muridarum plasmid revisited : new insights into growth kinetics [version 1; referees: 2 approved, 1 approved with reservations], Wellcome Open Res. 3 (2018), https://doi.org/10.12688/wellcomeopenres.13905.1.

[8] M.M. Cossé, R.D. Hayward, A. Subtil, One face of Chlamydia trachomatis: the infectious elementary body, 35–58, https://doi.org/10.1007/82_2016_12, 2016.

[9] S.S. Witkin, E. Minis, A. Athanasiou, J. Leizer, I.M. Linhares, Chlamydia trachomatis: the persistent pathogen, Clin. Vaccine Immunol. 24 (2017), https://doi.org/10.1128/CVI.00203-17.

[10] L. Wang, Y. Hou, H. Yuan, H. Chen, The role of tryptophan in Chlamydia trachomatis persistence, Front. Cell. Infect. Microbiol. 12 (2022), https://doi.org/10.3389/fcimb.2022.931653.

[11] M.R. Brockett, G.W. Liechti, Persistence alters the interaction between Chlamydia trachomatis and its host cell, Infect. Immun. 89 (2021), https://doi.org/10.1128/IAI.00685-20.

[12] M. Di Pietro, S. Filardo, S. Romano, R. Sessa, Chlamydia trachomatis and Chlamydia pneumoniae interaction with the host: latest advances and future prospective, Microorganisms 7 (2019), https://doi.org/10.3390/microorganisms7050140.

[13] M.E. Panzetta, R.H. Valdivia, H.A. Saka, Chlamydia persistence: a survival strategy to evade antimicrobial effects in-vitro and in-vivo, Front. Microbiol. 9 (2018), https://doi.org/10.3389/fmicb.2018.03101.

[14] G. Byrne, Chlamydial treatment failures: a persistent problem? J. Eur. Acad. Dermatol. Venereol. 15 (2001) https://doi.org/10.1046/j.1468-3083.2001.00343.x, 381–381.

[15] M.C. Jendro, T. Deutsch, B. Körber, L. Köhler, J.G. Kuipers, B. Krausse-Opatz, J. Westermann, E. Raum, H. Zeidler, Infection of human monocyte-derived macrophages with *Chlamydia trachomatis* induces apoptosis of T cells: a potential mechanism for persistent infection, Infect. Immun. 68 (2000) 6704–6711, https://doi.org/10.1128/IAI.68.12.6704-6711.2000.

[16] J. Dockterman, J. Coers, Immunopathogenesis of genital *Chlamydia* infection: insights from mouse models, Pathog. Dis. 79 (2021), https://doi.org/10.1093/femspd/ftab012.

[17] R. He, C.A. Torres, Y. Wang, C. He, G. Zhong, Type-I interferon signaling protects against Chlamydia trachomatis infection in the female lower genital tract, Infect. Immun. 91 (2023), https://doi.org/10.1128/iai.00153-23.

[18] K. Stelzner, N. Vollmuth, T. Rudel, Intracellular lifestyle of Chlamydia trachomatis and host–pathogen interactions, Nat. Rev. Microbiol. 21 (2023) 448–462, https://doi.org/10.1038/s41579-023-00860-y.

[19] A. Mayavannan, E. Shantz, I.D. Haidl, J. Wang, J.S. Marshall, Mast cells selectively produce inflammatory mediators and impact the early response to Chlamydia reproductive tract infection, Front. Immunol. 14 (2023), https://doi.org/10.3389/fimmu.2023.1166068.

[20] G. Kak, M. Raza, B.K. Tiwari, Interferon-gamma (IFN-γ): exploring its implications in infectious diseases, Biomol. Concepts 9 (2018) 64–79, https://doi.org/10.1515/bmc-2018-0007.

[21] J.D. Helble, R.J. Gonzalez, U.H. von Andrian, M.N. Starnbach, Gamma interferon is required for *Chlamydia* clearance but is dispensable for T cell homing to the genital tract, mBio 11 (2020), https://doi.org/10.1128/mBio.00191-20.

[22] T.W. Cotter, K.H. Ramsey, G.S. Miranpuri, C.E. Poulsen, G.I. Byrne, Dissemination of Chlamydia trachomatis chronic genital tract infection in gamma interferon gene knockout mice, Infect. Immun. 65 (1997) 2145–2152, https://doi.org/10.1128/iai.65.6.2145-2152.1997.

[23] M. Johansson, K. Schön, M. Ward, N. Lycke, Genital tract infection with Chlamydia trachomatis fails to induce protective immunity in gamma interferon receptor-deficient mice despite a strong local immunoglobulin A response, Infect. Immun. 65 (1997) 1032–1044, https://doi.org/10.1128/iai.65.3.1032-1044.1997.

[24] L.L. Perry, K. Feilzer, H.D. Caldwell, Immunity to Chlamydia trachomatis is mediated by T helper 1 cells through IFN-gamma-dependent and -independent pathways, J. Immunol. 158 (1997) 3344–3352.

[25] W.L. Beatty, G.I. Byrne, R.P. Morrison, Morphologic and antigenic characterization of interferon gamma-mediated persistent Chlamydia trachomatis infection in vitro, Proc. Natl. Acad. Sci. USA 90 (1993) 3998–4002, https://doi.org/10.1073/pnas.90.9.3998.

[26] I. Tietzel, C. El-Haibi, R.A. Carabeo, Human guanylate binding proteins potentiate the anti-Chlamydia effects of interferon-γ, PLoS One 4 (2009) e6499, https://doi.org/10.1371/journal.pone.0006499.

[27] S. Filardo, M. Di Pietro, F. Frasca, F. Diaco, M. Scordio, G. Antonelli, C. Scagnolari, R. Sessa, Potential IFNγ modulation of inflammasome pathway in Chlamydia trachomatis infected synovial cells, Life 11 (2021) 1359, https://doi.org/10.3390/life11121359.

[28] K.Y. Fung, N.E. Mangan, H. Cumming, J.C. Horvat, J.R. Mayall, S.A. Stifter, N. De Weerd, L.C. Roisman, J. Rossjohn, S.A. Robertson, J.E. Schjenken, B. Parker, C. E. Gargett, H.P.T. Nguyen, D.J. Carr, P.M. Hansbro, P.J. Hertzog, Interferon-ε protects the female reproductive tract from viral and bacterial infection, Science 339 (2013) (1979) 1088–1092, https://doi.org/10.1126/science.1233321.

[29] F.-R. Zhao, W. Wang, Q. Zheng, Y.-G. Zhang, J. Chen, The regulation of antiviral activity of interferon epsilon, Front. Microbiol. 13 (2022), https://doi.org/10.3389/fmicb.2022.1006481.

[30] P. Hermant, C. Francius, F. Clotman, T. Michiels, IFN-E is constitutively expressed by cells of the reproductive tract and is inefficiently secreted by fibroblasts and cell lines, PLoS One 8 (2013), e71320, https://doi.org/10.1371/journal.pone.0071320.

[31] A. Pierangeli, M. Gentile, G. Oliveto, F. Frasca, L. Sorrentino, L. Matera, R. Nenna, A. Viscido, M. Fracella, L. Petrarca, G. D'Ettorre, G. Ceccarelli, F. Midulla, G. Antonelli, C. Scagnolari, Comparison by age of the local interferon response to SARS-CoV-2 suggests a role for IFN-ε and -ω, Front. Immunol. 13 (2022) https://doi.org/10.3389/fimmu.2022.873232.

[32] C.A. DeCarlo, A. Severini, L. Edler, N.G. Escott, P.F. Lambert, M. Ulanova, I. Zehbe, IFN-κ, a novel type I IFN, is undetectable in HPV-positive human cervical keratinocytes, Lab. Invest. 90 (2010) 1482–1491, https://doi.org/10.1038/labinvest.2010.95.

[33] S.L. Day, I.A. Ramshaw, A.J. Ramsay, C. Ranasinghe, Differential effects of the type I interferons α4, β, and ε on antiviral activity and vaccine efficacy, J. Immunol. 180 (2008) 7158–7166, https://doi.org/10.4049/jimmunol.180.11.7158.

[34] R. Sessa, M. Di Pietro, F. De Santis, S. Filardo, R. Ragno, L. Angiolella, Effects of Mentha suaveolens essential oil on Chlamydia trachomatis, BioMed Res. Int. 2015 (2015), https://doi.org/10.1155/2015/508071.

[35] R. Sessa, M. Di Pietro, F. De Santis, S. Filardo, R. Ragno, L. Angiolella, Effects of *Mentha suaveolens* essential oil on *Chlamydia trachomatis*, BioMed Res. Int. 2015 (2015) 1–7, https://doi.org/10.1155/2015/508071.

[36] P. Kumar, A. Nagarajan, P.D. Uchil, Analysis of cell viability by the MTT assay, Cold Spring Harb. Protoc. 2018 (2018), https://doi.org/10.1101/pdb.prot095505 pdb.prot095505.

[37] C. Tasker, S. Subbian, P. Gao, J. Couret, C. Levine, S. Ghanny, P. Soteropoulos, X. Zhao, N. Landau, W. Lu, T.L. Chang, IFN-ε protects primary macrophages against HIV infection, JCI Insight 1 (2016), https://doi.org/10.1172/jci.insight.88255.

[38] A. Garcia-Minambres, S.G. Eid, N.E. Mangan, C. Pade, S.S. Lim, A.Y. Matthews, N. A. Weerd, P.J. Hertzog, J. Mak, Interferon epsilon promotes HIV restriction at multiple steps of viral replication, Immunol. Cell Biol. 95 (2017) 478–483, https://doi.org/10.1038/icb.2016.123.

[39] S.L. Day, I.A. Ramshaw, A.J. Ramsay, C. Ranasinghe, Differential effects of the type I interferons α4, β, and ε on antiviral activity and vaccine efficacy, J. Immunol. 180 (2008) 7158–7166, https://doi.org/10.4049/jimmunol.180.11.7158.

[40] R.C. Coldbeck-Shackley, O. Romeo, S. Rosli, L.J. Gearing, J.A. Gould, S.S. Lim, K. H. Van der Hoek, N.S. Eyre, B. Shue, S.A. Robertson, S.M. Best, M.D. Tate, P. J. Hertzog, M.R. Beard, Constitutive expression and distinct properties of IFN-epsilon protect the female reproductive tract from Zika virus infection, PLoS Pathog. 19 (2023), e1010843, https://doi.org/10.1371/journal.ppat.1010843.

[41] J.W. Mungin, X. Chen, B. Liu, Interferon epsilon signaling confers attenuated Zika replication in human vaginal epithelial cells, Pathogens 11 (2022) 853, https://doi.org/10.3390/pathogens11080853.

[42] Y. Xi, S.L. Day, R.J. Jackson, C. Ranasinghe, Role of novel type I interferon epsilon in viral infection and mucosal immunity, Mucosal Immunol. 5 (2012) 610–622, https://doi.org/10.1038/mi.2012.35.

[43] S. Filardo, M.D. Pietro, G. Tranquilli, M.A. Latino, N. Recine, M.G. Porpora, R. Sessa, Selected immunological mediators and cervical microbial signatures in women with Chlamydia trachomatis infection, mSystems 4 (2019), https://doi.org/10.1128/mSystems.00094-19.

[44] N.M. Bourke, S.L. Achilles, S.U.-S. Huang, H.E. Cumming, S.S. Lim, I. Papageorgiou, L.J. Gearing, R. Chapman, S. Thakore, N.E. Mangan, S. Mesiano, P.J. Hertzog, Spatiotemporal regulation of human IFN-ε and innate immunity in the female reproductive tract, JCI Insight 7 (2022), https://doi.org/10.1172/jci.insight.135407.

[45] C. Scagnolari, B. Caputo, G. Rezza, G. Antonelli, Antiviral activity of the combination of interferon and ribavirin against chikungunya virus: are the results conclusive? JID (J. Infect. Dis.) (2016) jiw579, https://doi.org/10.1093/infdis/jiw579.