The effect of IFN-γ on *Chlamydia trachomatis* growth in human Fallopian tube epithelial cells

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Introduction

An ectopic pregnancy (EP) is a pregnancy implanted outside the uterus, most commonly in the Fallopian tube (FT). It represents 1-2% of pregnancies, and is a leading cause of maternal morbidity and mortality worldwide. Past exposure to genital *Chlamydia trachomatis* (Ct) infection is a major risk factor (Jurkovic and Wilkinson, 2011) but the mechanism by which Ct leads to tubal implantation is not understood; not appearing to be a direct consequence of tissue destruction by the organism.

Objective

To establish an *in vitro* model of IFN-γ induced persistent Ct infection in a human FT epithelial cell line.

Methods

Cell culture and Ct infection

Human immortalised FT epithelial cells (OE-E6/E7) were cultured and infected with Ct at multiplicities of infection (MOI: ratio of host cells to Ct) of 1, 0.1 and 0.01. IFN-γ was added at increasing concentrations of 2.5, 5, 10, 20 and 40 ng/ml (n=4). Cells were harvested and fixed with methanol after 72 hours.

Detection and quantification of inclusions

Wells containing chlamydial inclusions were incubated with a mouse monoclonal anti-chlamydial lipopolysaccharide antibody and a biotinylated goat anti-mouse antibody. Detection was achieved using a streptavidin-biotin antigen detection system (DAB). Cells were counterstained with haematoxylin. Inclusions were identified visually under a microscope. The average inclusion count per mm^2 was calculated using 5 fields at predetermined locations and divided by the field area to obtain the number of inclusions per mm^2.

Data collection and statistical analysis

Results were analysed using repeated measures one-way ANOVA followed by non-parametric Dunn's multiple comparisons test, comparing untreated with IFN-γ-treated cells.

Results

OE-E6/E7 cells could be infected with Ct and inclusion numbers observed decreased with lower MOIs (Fig 1 and 2), reflecting a dose-dependent relationship. A significant decrease in inclusion number was observed with the addition of 10 ng/ml of IFN-γ. 40 ng/ml of IFN-γ resulted in a further decrease in inclusions, although this difference was less marked (Fig 3 and 4). Similar trends were observed at MOIs of 1 and 0.1, with a more drastic decrease in inclusion numbers at MOI of 1 due to a larger inclusion number in the negative control. From the inhibition of Ct growth in response to IFN-γ, we can infer that OE-E6/E7 cells possess an IFN-γ receptor that mediates this response.

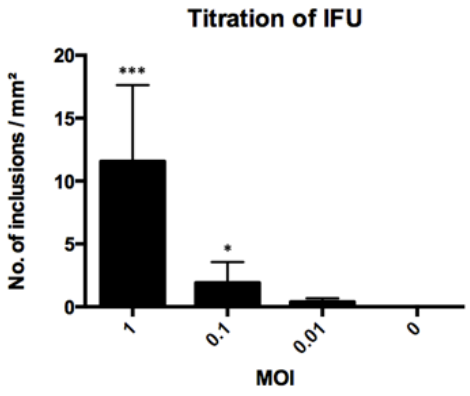

Fig 1. Differences by Dunn's multiple comparisons test between cells with varying MOIs were observed to be significant at $P < 0.05$ (*) and $P < 0.001$ (***).

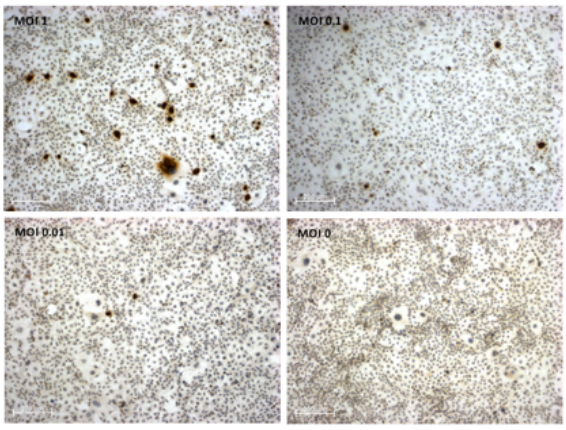

Fig 2. IFU titration with varying MOIs.

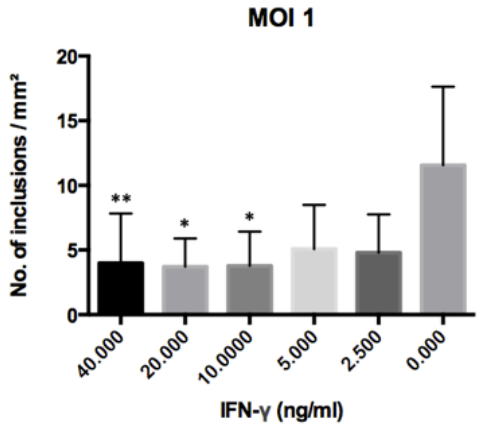

Fig 3. Differences by Dunn's multiple comparisons test between IFN-γ-treated vs untreated cells were observed to be significant at $P < 0.05$ (*) and $P < 0.01$ (**).

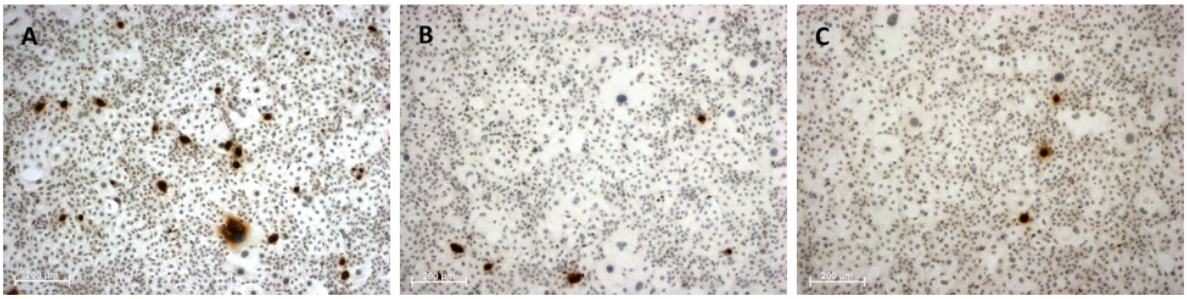

Fig 4. DAB staining of Ct inclusions grown without IFN-γ (A), with 10 ng/ml IFN-γ (B) and with 40 ng/ml IFN-γ (C).

Discussion

We have shown that IFN-γ is able to restrict Ct growth in the human FT epithelium. This suggests that the human FT epithelium contains receptors for IFN-γ, which can bind to IFN-γ and mediate an inhibitory effect on Ct proliferation.

As can be seen from Fig. 3, the graph lacks the typical sigmoidal shape expected from such experiments. This is likely to be due to excessive amounts of IFN-γ. Smaller intervals between 0 and 2.5 ng/ml of IFN-γ may have been more effective in charting the effect of IFN-γ dosage and establishing persistence. (Beatty, Byrne and Morrison 1993)

Conclusion

The OE-E6/E7 cell line permits the establishment of Ct infection. IFN-γ was shown to be effective in restricting chlamydial growth in human FT epithelial cells, as seen by markedly reduced Ct inclusions. However, further studies at lower doses of IFN-γ are required to elucidate a dose-response relationship and achieve the appropriate IFN-γ dose necessary to induce persistence. This will more closely mimic latent Ct infection and better function as an in vitro model of Ct persistence in the FT epithelium.

References

Beatty WL, Byrne GI, Morrison RP. Morphologic and antigenic characterization of interferon gamma-mediated persistent Chlamydia trachomatis infection in vitro. Proc Natl Acad Sci USA. 1993; 90(9): 3998-4002.

Jurkovic D, Wilkinson H. Diagnosis and management of ectopic pregnancy. BMJ 2011; 342:d3397.