

PWE-039

**PERSISTENT SURVIVAL OF LUMINESCENT  
MYCOBACTERIUM AVIUM SUBSPECIES  
PARATUBERCULOSIS IN PERIPHERAL BLOOD  
MONOCYTES FROM BOTH CROHN'S DISEASE  
AND HEALTHY CONTROLS IS ASSOCIATED WITH  
DIFFERENTIAL CYTOKINE RESPONSE**

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**Introduction** Abnormal intestinal macrophage function which could allow persistence of candidate bacterial triggering agents such as *E coli* and *Mycobacterium avium* subspecies *paratuberculosis* (MAP), has been implicated in the pathogenesis of Crohn's disease (CD).<sup>1</sup> Prolonged survival of *E coli* in CD-derived peripheral blood monocytes (PBM) has been demonstrated,<sup>2</sup> however current methods of determining MAP infection are difficult and time-consuming. The application of a luminescent MAP strain in a novel rapid technique may improve survival assays.<sup>3</sup>

**Aims** Determine the sensitivity and accuracy of a method to quantify luminescent MAP and develop an intracellular survival assay. Monitor cytokine profiles induced during MAP infection in CD and healthy controls (HC).

**Methods** Quantitative real-time PCR (qRT PCR) was used to calibrate a standard curve of luminescence from MAP 'Lux' 19 698 strain. Monocytes from CD and HC were isolated and infected with MAP lux 19 698 (multiplicity of infection 4:1) for up to 4 weeks. Luminescence was recorded at intervals to determine killing activity compared to medium only controls. Intracellular uptake was visualised using an anti-MAP antibody and confocal microscopy. Cytokine release (TNF $\alpha$ , IL10, IL6 and IL23) from PBM was measured using ELISA pre and 24 h post infection.

**Results** A close linear relationship ( $R^2 = 0.9999$ ) was seen between luminescence and number of MAP organisms by qRT PCR. Sensitivities were also similar. Monocytes from 8 CD patients and 7 HC infected with MAP lux 19 698 assayed over a mean of 15 days (range 8 to 27) showed some killing during the experiment time course. However a majority of MAP survived intracellularly (visualised by confocal microscopy) in all samples and the degree of killing was not significantly different between CD and HC. Cytokine profiling showed only a proportion of all subject's (CD or HC) PBM responded to MAP infection. Of these responders, there was a difference in cytokine profiles between CD and HC and in particular there was a strong trend to greater IL6 response in CD.

**Conclusion** This study provides initial validation for a luminescent MAP intracellular survival assay. Consistent, persistent survival of MAP was demonstrated in both CD and controls. Alterations in cytokine responses to MAP infection between CD and normals suggest that this agent is perceived and processed differently by CD monocytes.

**Competing interests** None.

## REFERENCES

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