DEMONSTRATION OF INTRACELLULAR MYCOBACTERIUM SPECIES IN CROHN'S DISEASE USING NOVEL TECHNOLOGIES

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INTRODUCTION

Mycobacterium avium paratuberculosis (MAP) has been implicated in the pathogenesis of Crohn's disease (CD) for a long time. Scientific rejection of this association has been influenced by variability of detection of MAP, and a perception of a lack of evidence of causation although Koch's postulates have been fulfilled[1]. Epidemiology[2] and histopathological observations also strongly support a role[3].

We describe the characteristics of an intracellular, cell-wall-deficient *Mycobacterium species*, (L form) *closely resembling MAP, that was* isolated with high frequency and accuracy from the blood of Crohn's disease patients

AIM

To detect and culture non-TB intracellular cell-wall-deficient (L form) bacteria, in dendritic cells and macrophages of CD patients before and during Anti-MAP treatment and in controls.

METHODS

Peripheral blood samples were stained with conventional Ziehl-Neelsen (ZN) stain after 8d and 30d incubation in a range of supplemented broths. Cells containing stainable intracellular bacteria were expressed as a ratio of total cells using x1000 oil immersion microscopy.

The Investigator staining the cells was blinded to the diagnosis.

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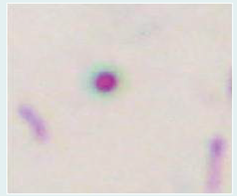
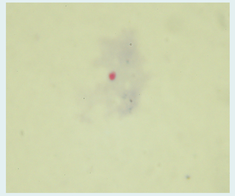
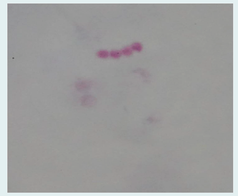
L Form Bacteria culture and detection images



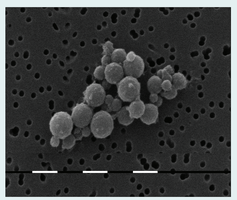
1. (ZN stain) A biofilm- shrouded L form, flanked by two bacilliary mycobacteria



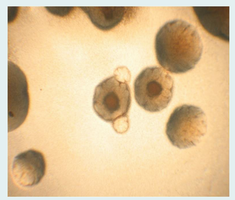
2. (ZN stain) A dormant mycobacterial L form producing biofilm in broth culture



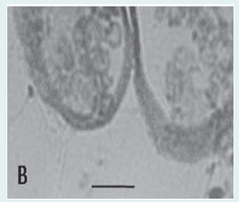
3. Binary fission and septate formation in broth culture of L forms



4. Scanning electron microscopy of a cluster of mycobacterial L forms

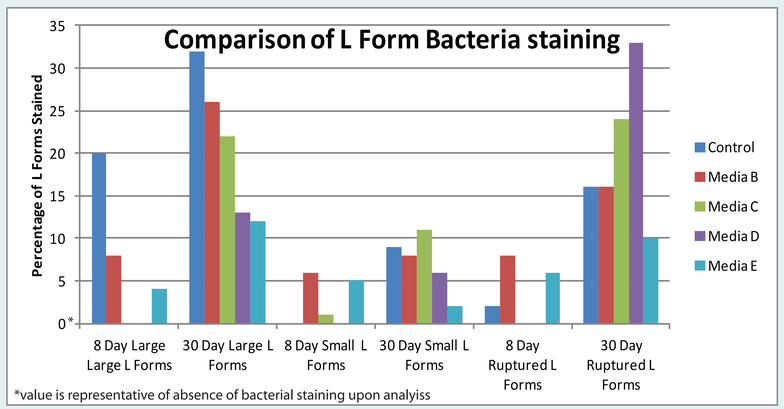


5. Solid medium culture of mycobacterial L forms ex blood culture



6. Transmission Electron Microscopy showing mycobacterial L form structure

Fig 1. Culture and stain of L forms on solid media



RESULTS

ZN staining of mycobacterial L forms was achieved in a subset of 80 individuals. CD was correctly identified in 64/68 (94%) and by 30 days the stained L forms had often multiplied to fill the macrophage.

In controls 11/12 had a low-level staining of spherules at 30 days with 5/12 correctly identified as normal due to the morphology and sparcity of the L forms. Only one normal individual did not exhibit L forms.

Later we cultured *extracellular* L forms on solid media (Fig 1). Probing the samples with *Mycobacterium* species-specific probes confirmed the presence of *Mycobacterium* species, which awaits further characterisation. Morphology of human L forms resembles that of Red Deer infected with MAP and blood of patients immunised with Bacille Calmette-Guerin.

Preliminary work with CD patients showing L forms indicates a relationship with MAP.

Anti-MAP antibiotics in CD patients profoundly suppressed growth of L forms but did not eradicate them.

CONCLUSIONS

1. We confirm presence of intracellular *Mycobacterium* species L forms in blood macrophages of CD patients and in a proportion of controls.
2. L form growth is suppressed with Anti-MAP antibiotics paralleling clinical improvement, which further supports a causal association.

REFERENCES

1.Segal AW et al Lancet 1976;2:219–221.
2.Gearry, R., Aitken, J., Roberts et al. J Gastroenterol and Hepatol 2005;20: 1943