

expression (MFI) of CD25+ cells was then monitored after two days by flow cytometry.

Results 1,25(OH)₂D₃ significantly increased CTLA4 MFI in both healthy and latent populations following stimulation with SEB ($p \leq 0.01$) or PPD ($P = 0.026, 0008$). 25(OH)D₃ also enhanced CTLA-4 expression in SEB cultures ($p \leq 0.01$). Induction of CTLA-4 was however reduced in PPD cultures (median 121) compared to SEB (median 360). Interestingly, the magnitude of CTLA-4 induction by 1,25(OH)₂D₃ or 25(OH)D₃ also differed for healthy and latent populations in response to SEB (1,25(OH)₂D₃ ($p = 0.01$) and 25(OH)D₃ ($p = 0.006$), with a similar trend in PPD stimulated cells ($p = 0.092$).

Conclusion The shift towards a T reg population as a result of vitamin D is blunted in latent TB compared to health. Differential response of memory cells in latent disease could account for this.

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S4 ANTI-INFLAMMATORY EFFECTS OF VITAMIN D ARE INFLUENCED MORE BY GENETIC BACKGROUND THAN MYCOBACTERIAL INFECTION

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G Hawthorne, H Mujakperuo, S Lax, L McGowan, H Kunst, D Thickett, A Turner. *School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, UK*

Introduction Although vitamin D has antimicrobial effects that may be beneficial in mycobacterial infection, supplementation trials have been disappointing [1]. Response to vitamin D is heterogeneous, perhaps due to genetic variants in the vitamin D axis (vitamin D binding protein; GC or vitamin D receptor; VDR). We aimed to assess stability and magnitude of anti-inflammatory effect of vitamin D in vitro in mycobacterial infection and health, and the influence of GC and VDR variants.

Methods Peripheral blood monocytes from healthy controls ($n = 20$) and patients with mycobacterial infection ($n = 8$) were cultured and incubated with LPS (100 ng/ml), 25(OH)D₃ (50 nmol) and 1,25(OH)₂D₃ (50 nmol). IL6 in cell supernatants was measured by ELISA. Taqman was used to type 4 SNPs: rs7041 & rs4588 (GC), rs2228570 & rs1544410 (VDR). The effect of the protein product of GC in culture was assessed by addition of plasma and subsequent Luminex array.

Results Change in IL6 between pairs of repeated cultures did not vary ($p = 0.21$), indicating stability of response to LPS. There was no difference between HCs and patients in response to vitamin D (both $p > 0.32$), however in both groups there were responders ($n = 7$ controls, $n = 5$ TB), in whom IL6 fell with addition of vitamin D (1,25D, $p = 0.001$; 25D, $p = 0.02$) and non-responders ($n = 11$ controls, $n = 3$ TB) in whom it did not.

Response to 1,25D was influenced by rs7041 (T allele; $p = 0.04$), and tended to associate with GC2 ($p = 0.06$). Response to 25D was not affected by genotype until plasma was added when GC haplotype appeared to have an effect. There was no influence of VDR SNPs.

Conclusion Anti-inflammatory effects of vitamin D are influenced by GC genotype in vitro, consistent with previous mouse work [2], however it is unclear whether this occurs in humans in vivo. Such effects are equally relevant in health and mycobacterial infection. Further work to characterise changes in LPS responsiveness following high dose vitamin D replacement is ongoing.

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S5 MOLECULAR IMMUNODIAGNOSIS OF TB INFECTION: A PILOT STUDY

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¹DW Connell, ¹A-K Reuschl, ¹C Wenden, ²M Witkowski, ³OM Kon, ²P Klennerman, ¹A Lavani. ¹Tuberculosis Research Unit, NHLI, Imperial College London, London, United Kingdom; ²NIHR Biomedical Research Centre, Peter Medawar Building, University of Oxford, Oxford, United Kingdom; ³Department of Chest and Allergy, St Mary's Hospital; Imperial College Healthcare NHS Trust, London, United Kingdom

Introduction Current interferon-gamma (IFN- γ) release assays (IGRAs) are not sufficiently sensitive to be used as a “rule-out” test for tuberculosis (TB). Antigen-specific gene expression of chemokines downstream of, and amplified by, IFN- γ might demonstrate such sensitivity, and can be performed with very small volumes of blood.¹

Here we assess the performance and sensitivity of two IFN- γ -dependent gene expression platforms in the peripheral blood mononuclear cells of individuals with and without TB.

Methods 23 individuals with active TB, 12 individuals with latent TB infection (LTBI), and 18 controls had simultaneous IFN- γ ELISpot assays and qRT-PCR of CXCL-9 and CXCL-10, following stimulation with the immunodominant antigens ESAT-6, CFP-10 and EspC (6 gene expression assays in total). Test performances of the 6 gene expression assays were compared to the ELISpot.

Results Gene expression of CXCL-9 and CXCL-10 was antigen specific, correlating well with each other and with the IFN- γ ELISpot (Spearman Rank Correlations ranging from $r = 0.648$ to 0.74). Gene expression of either was not significantly different between those with active TB and LTBI.

Receiver-operating characteristic curves indicated good test performances for all the gene expression assays (AUC ranging from 0.918 to 0.959) and agreements between the ELISpot and gene expression platforms was good (κ statistic ranging from 0.403–0.496).

After constructing cut-offs for sensitivity for individual antigens, with cut-offs for specificity matching or exceeding that of the IFN- γ ELISpot, the sensitivity of TB diagnosis with gene expression was superior to ELISpot in 5 out of the 6 assays, although these differences were not statistically significant. Sensitivity was equivalent when antigens were combined, as in the commercially available T-Spot[®].TB.

Conclusions These pilot data indicate that gene expression of IFN- γ -dependent cytokines is a robust, sensitive and specific method of TB diagnosis, and carries potential to be a more sensitive platform than the current gold standard – IFN- γ ELISpot. Larger studies with appropriate power are now required in similar populations to investigate this approach definitively.

References

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Targets for asthma therapy

S6 THE PHENOTYPE TRIAL: INHALED CORTICOSTEROID DOSE-RESPONSE USING DOMICILIARY EXHALED NITRIC OXIDE IN PERSISTENT ASTHMA

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WJ Anderson, PM Short, PA Williamson, BJ Lipworth. *University of Dundee, Dundee, United Kingdom*

Background International guidelines advocate a standard approach to asthma management for all despite its heterogeneity. ‘Personalised’ treatment for inflammatory asthma phenotypes confers superior benefits. We wished to evaluate dose-response to inhaled corticosteroids (ICS) in asthmatics with an elevated exhaled nitric oxide (FeNO) phenotype using domiciliary measurements.