BSG INFLAMMATORY BOWEL DISEASE SECTION SYMPOSIUM

OC-073 **NOD2 REGULATION OF MICRORNAS**

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O Brain,[1,*] P Allan,[1] T Pichulik,[2] E Khatamzas,[2] P Simpson,[1] D Jewell,[3] A Simmons[1] *[1]Translational Gastroenterology Unit, John Radcliffe Hospital, Oxford, UK; [2]HIU, WIMM, John Radcliffe Hospital, Oxford, UK; [3]Dept of Gastroenterology, John Radcliffe Hospital, Oxford, UK*

Introduction Crohn's Disease (CD) is thought to arise both from defects in the gut mucosal barrier and from a dysregulated Th1/ Th17 immune response to commensal gut flora.
CARD15 polymorphisms confer susceptibility to terminal ileal CD. Nod2 responds to muramyl dipeptide (MDP) leading to NF-κB activation and release of pro-inflammatory cytokines. CD-associated Nod2 mutations are predominantly loss-of-function, and it is uncertain how this predisposes to a pro-inflammatory disease. Published data have revealed Nod2 cross-talks with toll-like receptors. This study investigatesv the role of microRNAs in regulating Nod2 signalling and Nod2/ TLR cross-talk.
MicroRNAs are short non-coding RNAs that prevent the translation of mRNA into protein and can negatively regulate innate immunity. Loss of these negative regulators might lower the threshold to the development of a pro-inflammatory state in mucosal tissue.
Aims (1) Identify microRNAs differentially expressed in human dendritic cells (DCs) following Nod2 and combined Nod2/ TLR, stimulation. (2) Determine the microRNA targets. (3) Determine the functional consequences of miRNA expression.
Methods Monocyte-derived DCs, expressing either WT or 1007fsinsC CARD15, were stimulated with MDP, Pam3CSK4 (TLR2 ligand), or Flagellin (TLR5 ligand) before lysis and extraction of RNA. MiRNA microarray analysis was conducted using Illumina miRNA microarrays. MiRNA expression was validated with quantitative PCR. Potential miRNA targets were identified using available algorithms and from published data. MiRNA mimic and antimir transfection were used to confirm targets by western blot, elisa and 3'UTR lucferase assay. Further targets and functional effects were determined by dual-colour Affymetrix cDNA array following miRNA mimic transfection into DCs. Functional consequence of loss of miRNA expression determined by intracellular bacterial killing assay, confocal microscopy, FACS analysis and T-cell proliferation assay.
Results Nod2 stimulation is required for expression of two miRNA clusters, in combination with either TLR2 or TLR5 triggering. MiRNA up-regulation is dependent on RIPK2 expression. DCs from patients homozygous for 1007fsinsC CARD15 fail to up-regulate these miRNA clusters. MiRNA cluster over-expression down-regulates the IL12-p40 subunit in human DCs at both mRNA and protein level. This subunit is a component of both IL-12 and IL-23 cytokines. IL-12p40 down-regulation can be 'rescued' in FS1007insC DCs by miRNA mimic expression.
Conclusion DCs from patients with CD-associated mutations of Nod2 fail to up-regulate two miRNA clusters. These clusters regulate a critical component of the Th1/ Th17 immune response. These findings may help link CARD15 polymorphisms and the pathogenesis of CD.
Competing interests None.
Keywords Crohn's disease, microRNA, NOD2.