

Juvenile systemic lupus erythematosus with a baseline high interferon signature associates with increased immune cell TLR7 expression and enhanced TLR7 dependent IFN α production.

Kate Webb¹, Gary Butler², Coziana Ciurtin¹, Hannah Peckham¹, Anna Radziszewska¹, Lucy R Wedderburn^{1,3}, Yiannis Ioannou¹

1. Arthritis Research UK Centre for Adolescent Rheumatology, UCL, UCLH and GOSH
2. Department of Paediatric and Adolescent Endocrinology, UCLH and UCL GOS Institute of Child Health
3. NIHR Biomedical Research Centre at GOSH

Introduction: Juvenile onset systemic lupus erythematosus (jSLE) is characterised by a type 1 interferon (IFN) transcription signature. It is reported that up to 80% of patients with jSLE of varying severity express a predominant IFN signature. IFN signature however, correlates with disease activity, and is abrogated by steroids. The prevalence of IFN signature in baseline disease with minimal steroid dose is not known. Investigating the IFN signature outside of a flare may provide insight into the true prevalence of high IFN signature disease, as well as underlying differences in disease pathogenesis, that may be masked by steroid or disease activity.

Objectives: To investigate the prevalence of high IFN signature in low disease activity jSLE, and to assess whether toll like receptor (TLR) 7 or 9 dependent IFN α production pathways differ between high and low IFN signature patients.

Methods: Blood was collected, with informed consent, from young healthy volunteers (n=24:10 female; 14 male, age=12-19) and young people with jSLE (n=29:20 female; 9 male, age=14-21). Clinical data, including disease activity score (SLEDAI), organ involvement and treatment, were recorded. Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll gradient centrifugation. RNA was extracted ex vivo, and assessed by Nanostring Plex Set. IFN score was calculated from counts of 5 IFN inducible genes (MX1+BST2+MCP1+ISG15+ IFIT1/5). A normal range was calculated using healthy controls only. Samples were classed as IFN positive (IFN+) if >2SD from the healthy mean. Separately, PBMC were stimulated with TLR7 agonist, R848, or TLR9 agonist, CPGODN2216, before assessing for secretion of IFN α and TNF α by luminex. Statistical analysis was performed using SPSS via univariable and multivariable linear regression.

Results: There was a significantly higher IFN score in jSLE than healthy controls (p=0.001). 14/29 (48.27%) jSLE patients were IFN+ vs 1/23 (0.04%) healthy controls (p=0.005). Patients with jSLE had an average SLEDAI of 3.0 (range=0-12), 20 (52%) were on prednisone, with a mean dose of 6.9mg/day. In patients with jSLE, the only autoantibody that significantly predicted IFN score was RNP (p=0.018). There was no association between any specific organ involvement and IFN score. Four patients with jSLE were B cell depleted, but there was no effect of B cell depletion on IFN score. There were no significant differences in clinical markers of disease activity (SLEDAI, CRP, ESR, C3, C4 and double stranded DNA antibodies) between patients with jSLE who were IFN+ or IFN-. Only jSLE patients who were IFN+ produced significantly more IFN α (p=0.046) and TNF α (p=0.031) than healthy controls after TLR7 stimulation. After TLR9 stimulation, IFN α production was decreased in jSLE compared to healthy controls, regardless of IFN score. Patients with jSLE who were IFN+ had significantly higher PBMC RNA expression of TLR7 than those who were IFN- (p=0.001), and healthy controls (p=0.04). Patients with jSLE who were IFN+ showed a significant decrease in TLR9 expression when compared to healthy

controls ($p=0.023$). We confirmed that TLR7 was not an IFN inducible marker, by assessing expression in healthy controls with and without IFN α pre-stimulation, which showed no significant difference.

Conclusion: In non-flaring patients with jSLE on low doses of steroid, approximately half are IFN signature positive. This is lower than reported in studies that include flaring patients. In young people with jSLE, IFN signature score associates with RNP positivity. PBMC samples from IFN+ patients produce more IFN α after TLR7 stimulation, and have a significantly higher expression of TLR7, along with a decrease in TLR9 expression compared to samples from patients with a negative IFN signature, and healthy controls.