>2.6-3.2 (low DAS), >3.2-5.0 (moderate DAS), and >5.1 (high DAS), respectively. ** $p<0.01$, *** $p<0.0001$ (Independent t-test and Kruskal Wallis test)

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SAT0008 IL-23 DRIVES PATHOGENIC TH17 CELLS THROUGH EPIGENETIC REGULATION BY STAT3 IN SLE PATIENTS

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Background: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by loss of self-tolerance and by broad immune dysregulation, which result in overproduction of autoantibody and cause severe inflammation in various organs⁽¹⁾. Although amplified mature Th17 cells are thought to be vital role in the development and progression of SLE, mechanism underlying their pathogenicity remain widely elusive in human⁽²⁾.

Objectives: To elucidate the mechanism of pathogenic Th17 cells expansion, we probe the phenotype of Th17 cells in lupus patients and underlying epigenetic modification mediated by cytokine-induced signal transducer and activators of transcription (STAT) family factors in vitro.

Methods: Human naive CD4⁺ T cells were cultured by Th17 polarizing condition (TGF- β , IL-6, and IL-1 β) for 5 days in vitro. To examine the role of specific cytokine in maturation of Th17 cells, we added various cytokines including IL-23 at the later time point (72hr). Expression of characteristic markers of pathogenic Th17 cell and STATs phosphorylation (p-STATs) were analyzed by flow cytometry and qPCR. The effect of baricitinib which is JAK1/JAK2 inhibitor on maturation of Th17 cell was investigated. Histone modifications were assessed by chromatin immunoprecipitation (ChIP)-PCR. To define the signals mediated by cytokine required for expansion of Th17 cell in SLE, Th17 phenotype and pSTATs were analyzed from blood samples of lupus patients by multi-color flow cytometry.

Results: In vitro, re-stimulation of Th17 cells with IL-23 markedly induced the characteristic markers of pathogenic Th17 cells such as ROR γ t and IL-17. IL-23 overwhelmingly activates the p-STAT3, not p-STAT4 for development of mature Th17 cells. IL-23-induced p-STAT3 was inhibited by baricitinib. In addition, proliferation of Th17 cells was suppressed by baricitinib in concentrate dependent manner. The loci of ROR γ t at STAT binding sites were marked by bivalent histone modifications. After IL-23 stimulation, STAT3 exclusively bound on ROR γ t gene loci supplemented by activating H3K4me3 and repressing H3K27me3 modifications. In memory Th17 cells, high proportion of IL-23R expression and STAT3/4 activation were identified from SLE patients compare with healthy individuals. Moreover, p-STAT3 was hypersensitively activated by IL-23 stimulation in memory Th17 cell only from lupus patients but not from healthy controls.

Conclusion: This study proves that IL-23 serves as a pivotal factor that drives expansion of pathogenic Th17 cells. IL-23-mediated STAT3 alter histone modification, resulting in inflammatory function of pathogenic Th17 cell that are characteristically expanded in patients with SLE. These findings could be one of the underlying mechanisms of pathogenesis of SLE and helpful evidence toward novel therapeutic targets for SLE.

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SAT0009 THE EFFECT OF FCGR3A POLYMORPHISM ON THE INITIAL DEPTH OF B-CELL DEPLETION BY RITUXIMAB, FUNCTIONAL NK-CELL MEDIATED KILLING AND CLINICAL RESPONSE IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Antibody-dependent cellular-mediated cytotoxicity (ADCC) is an important effector mechanism that contributes to the depletion of therapeutic antibody-opsonised cells *in vivo*. Rituximab (RTX) is an anti-CD20 antibody with a native IgG1 Fc, which crosslinks Fc receptors (Fc γ Rs) expressed on immune effector cells. NK cells, which express Fc γ RIIIa, are believed to play an important role with less well-characterised contribution from monocytes/macrophages and neutrophils. A genetic variant in *FCGR3A* (158V) with an increased affinity for IgG1 has been reported to correlate with clinical response to RTX in RA[1]. Data in SLE are limited as the *FCGR* genetic locus is characterised by structural variation making genotyping challenging.

Objectives: To assess the effect of *FCGR3A*-F158V polymorphism on (i) the initial depth of B-cell depletion by RTX; (ii) its functional effect on NK cell-mediated killing (iii) clinical response and (iv) 10-year RTX retention for the treatment of SLE.

Methods: A prospective longitudinal study was conducted in RTX-treated SLE patients in Leeds. B-cells were measured at baseline and 6 weeks using highly sensitive flow cytometry. Complete depletion was defined as total B-cell count < 0.0001 $\times 10^9$ /L. A major clinical response (MCR) was defined as improvement of all active BILAG-2004 domains to grade C/ better and no A/B flare at 6 months. We measured qualitative polymorphism for *FCGR3A* using a multiplex ligation-dependent probe amplification as previously described[2]. Median expression of Fc γ RIIIa on NK cells (CD3-CD56+CD16+) and NK cell degranulation (CD107a) in the presence of RTX-coated Raji cells (B-lineage cell line) were assessed using flow cytometry.

Results: 85 SLE patients with at least 2 $\times$ BILAG B were studied [Female: 82(96%); mean age (SD): 40(14) years; 60/85(71%) on concomitant DMARDs; ANA positive: 100%]. In cycle 1 RTX, 64/85(75%) achieved BILAG response [major=36%; partial=39%]. Both complete depletion and MCR were associated with carriage of the *FCGR3A*-158V allele (158FV or 158VV), OR 2.73 (95% CI 1.13-6.58) and 3.06 (95% CI 1.19-7.58) vs 158FF, respectively.

Patients who had complete B-cell depletion post-RTX had higher level of Fc γ RIIIa on NK cells vs incomplete depletion; $p=0.006$. Comparing patients with *FCGR3A*-158V allele carriage vs FF genotype, there was a trend to higher expression of CD3-CD56+CD16+ in the former $p=0.073$. Moreover, degranulation as defined by ratio between %CD107a+ NK cell in the presence of Raji cells + RTX and %CD107a+ NK cell in Raji cells only, was higher in the V allele carriers vs FF genotype; $p=0.024$.

Over 10-year follow-up, 27/85 (31.8%) had stopped RTX [primary inefficacy=7; secondary inefficacy=8; HACA=10; death=2]. *FCGR3A*-158FF genotype was associated with increased risk of RTX discontinuation; OR 2.36 (95% CI 1.08-5.18) after adjusting for age and DMARDs.

Conclusion: An ADCC-enhancing Fc γ RIIIa variant was associated with initial complete B-cell depletion and MCR in RTX-treated SLE patients. This was supported by functional data on NK cell-mediated cytotoxicity and 10 year outcomes. These results elucidate one mechanism of resistance to rituximab in SLE and may guide development of more effective B-cell targeted strategies. With further validation, this polymorphism may be used to stratify SLE patients for therapy.

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