Background and aims Dectin-1 is a c-type lectin like receptor that signals via syk and is involved in anti-fungal immunity. Dectin-1 was found to trigger experimental inflammatory arthritis, and likely play a role in the pathogenesis of some autoimmune diseases. This study aimed to examine dectin-1 expression and function of circulating CD14+ monocytes and monocyte-derived dendritic cells (MDDCs) in patients with systemic lupus erythematosus (SLE).

Methods SLE patients with active and inactive diseases and healthy subjects were recruited. Dectin-1 agonists including curdlan, zymosan and toll-like receptor agonists Pam3CSK4 (TLR2) and LPS (TLR4) were used to stimulate monocytes and/or MDDCs. Dectin-1, ROS and phosphorylated-syk (p-Syk) were measured by flow cytometry. Cytokine profile was measured by and multi-bead immunoassay.

Results Dectin-1 expressing monocytes was significantly lower in active SLE patients compared to inactive patients and healthy controls. The absolute count of dectin-1 expressing monocytes correlated significantly and inversely with SLEDAL, anti-dsDNA antibody level and C4. Despite this, ROS production upon stimulation by dectin-1 agonists was comparable. Stimulation of dectin-1 led to activation and maturation of MDDCs. SLE MDDCs showed higher p-Syk activation compared to normal MDDCs upon dectin-1 stimulation. Curdlan-stimulated MDDCs produced higher levels of IL-1β, IL-23 and TNF-α. Adding TLR2 agonist to curdlan, SLE MDDCs produced significantly higher level of IL-1β compared to normal MDDCs.

Conclusions Active SLE patients had significantly lower circulating dectin-1 expressing monocytes which produced comparable level of ROS compared to inactive patients and healthy subjects. Dectin-1 agonists led to significantly higher Th17 promoting cytokines upon co-stimulation with TLR2 in SLE MDDCs.

Abstracts

349 TMEM173/STING IS CRUCIAL FOR LUPUS DEVELOPMENT IN FCGR2B-DEFICIENCY MICE

1P Prisut*1, A Thim-Uam1, M Tansuk1, B Wongprom1, A Leelawaranichkul1, S Paludan, 2J Wang*, 11J Marken1, 3K Cerasi11, M Li, 2K Elkon, 1K Zeng, 1N Gilh1a, 1Peking Union Medical College Hospital, Rheumatology, Beijing, China; 2University of Washington, Rheumatology, Seattle, USA; 3Benaroya Research Institute at Virginia Mason, Translational Research Program, Seattle, USA

Background and aims Type I interferon is one of the most critical cytokines involving in lupus pathogenesis. The activation of endosomal nucleic acid sensors leading to type I IFN production empowers the lupus phenotypes in several mouse models. The signalling of cytosolic DNA sensor also induces type I IFN production and the role in lupus disease are not clear. Stimator of interferon genes (Sting), also known as transmembrane protein 173 (TMEM173) and MPYS/MITA/ERIS, is a cytosolic DNA sensor which recognised cyclic di-GMP and subsequently stimulated type I interferon production. The FcgR2b-deficient mice develop spontaneous lupus phenotypes which are splenomegaly, the presence of anti-nuclear antibodies (ANA) and fatal glomerulonephritis. The polymorphisms of FCGR2B associates with the increases of lupus susceptibility in human. The goal of the study is to identify the role of Sting in lupus mouse model.

Methods The Sting-deficient mice were bred with the FcgR2b-deficient mice to create double deficient mice and control littermates. The mice were analysed for survival rate, autoantibodies production, severity of pathology, gene expression profiles, and immunophenotypes.

Results In the absence of Sting, the FcgR2b-deficient mice survived longer and the level of ANA and anti-dsDNA antibody considerably reduced. The glomerulonephritis in the double-deficient mice also ameliorated. The expression of interferon inducible genes such as Cxcl10, Mx1, and Ifi5 in the kidneys of the double-deficient mice was significantly lower than the FcgR2b-deficient mice.

Conclusions Sting-mediated signalling pathway plays the substantial role in lupus pathogenesis of the FcgR2b-deficient mice. Blocking Sting function may be the advantage for treatment in lupus patients.

350 TOLL-LIKE RECEPTOR 7 SIGNALLING DRIVES TRANSITIONAL B CELLS EXPANSION AND AUTOANTIBODY PRODUCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS

1T Wang*, 2J Marken, 3K Cerasi, 1M Li, 2K Elkon, 1X Zeng, 1N Gilh, 1Peking Union Medical College Hospital, Rheumatology, Beijing, China; 2University of Washington, Rheumatology, Seattle, USA; 3Benaroya Research Institute at Virginia Mason, Translational Research Program, Seattle, USA

Background and aims Toll-like receptor 7 (TLR7) has been implicated in B cell activation and the generation of pathogenic autoantibodies. Newly-formed transitional (TR) B cells are enriched in autoreactive specificities and are increased in some SLE patients. This study was undertaken to examine a possible link between the TR B cells expansion/activation and TLR7 levels in SLE.

Methods PBMCs were collected from SLE patients and healthy donors and analysed for the expression of TLR7, TLR9 and IFN-responsive genes by RT-PCR. The frequencies of B cell populations were analysed by flow cytometry. BAFF titers were analysed by ELISA. TLR7 variant rs3853839 (C/G) was detected by Taqman 5’-allele discrimination assay. TR B cells were primed with IFNα and stimulated with TLR7 ligands in vitro.

Results High expression levels of TLR7 in SLE patients positively correlated with IFN signature and disease activity, but not with BAFF titers. SLE patients with high levels of TLR7 (TLR7hi group) showed an expansion of CD19+CD38hiCD24hiCD10− TR B cells. Overall, frequencies of TR B cells positively correlated with the levels of TLR7, but not TLR9. SLE patients, carrying a risk G allele, had increased TLR7 expression and TR cell frequencies, compared to non-risk allele carriers. TLR7hi SLE patients showed increased autoantibody titers and skewed towards Sm/RNP antigens. Upon IFNα priming, TR B cells up-regulated TLR7 and differentiated into plasmablasts in response to TLR7-ligand stimulation.

Conclusions Our findings suggest that dysregulation of TLR7 in SLE might drive the expansion and promote the activation of TR B cells, which might be a source of autoantibodies.
Background and aims C-type lectin domain family 16 member A (CLEC16A) has been associated with autoimmune diseases such as systemic lupus erythematosus, multiple sclerosis and type 1 diabetes in various genome-wide association studies. Subsequent studies revealed that mouse/human CLEC16A and its Drosophila homolog endosomal maturation defective isoform A (EMA) are involved in different aspects of autophagy, the regulated degradation of cellular components that are in excess or dysfunctional. Crosstalk between autophagy and inflammasome activity of innate immune responses has been reported and inflammasomes are activated in various autoimmune diseases. We thus sought to investigate the role of CLEC16A in inflammasome pathway in this study.

Methods Functional genetic studies of CLEC16A in NLRP3 and AIM2 inflammasome pathways using monocyte-derived macrophages isolated from peripheral blood mononuclear cells of healthy individuals were performed. Results During induction of NLRP3 inflammasome pathway by nigericin, a knockdown of CLEC16A using specific siRNAs inhibited secretion of interleukin-1β (IL-1β), an inflammasome pathway effector. Its secretion during AIM2 inflammasome induction by intracellular dsDNA poly(dA:dT) however was not affected in the siCLEC16A group. The induction of NLRP3 mRNA level upon lipopolysaccharide stimulation was suppressed in the siCLEC16A group. No significant changes in mRNA levels was observed in other selected genes of NLRP3 inflammasome pathway, namely the adaptor protein ASC, interleukin-1 converting enzyme caspase-1 and precursor pro-IL-1β.

Conclusions These data suggest that CLEC16A regulates NLRP3 but not AIM2 inflammasome pathway and affects IL-1β secretion in part via NLRP3 level. The mechanism involved and its association with autoimmune diseases such as systemic lupus erythematosus remains to be elucidated.