### 336 DEFECT OF BDH2 CONTRIBUTES TO DNA HYPOMETHYLATION IN CD4+ T CELLS OF SYSTEMIC LUPUS ERYTHEMATOSUS

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**Background and aims** A lot of evidences have confirmed that genomic DNA hypomethylation play an important role in the pathogenesis of systemic lupus erythematosus (SLE). However, the mechanism of DNA hypomethylation in lupus CD4+ T cells remain unclear. Previous study showed that depletion of the mammalian siderophore by inhibiting expression of 3-OH butyrate dehydrogenase (BDH2) results in abnormal accumulation of intracellular iron and mitochondrial iron deficiency in cultured mammalian cells. In this study, we investigate whether BDH2 is involved in regulating DNA hypomethylation in CD4+ T cells of SLE.
**Methods** 20 SLE patients and 20 healthy controls were recruited. CD4+ T cells were isolated by magnetic beads. All patients fulfilled at least 4 of the SLE classification criteria of ACR. mRNA and protein levels were detected by real-time PCR and western blot. Global DNA methylation level was measured by Global DNA Methylation Assay–LINE-1 kit. CD4 + T cells were transfected by nucleofector.
**Results** Compared with normal controls, BDH2 mRNA and protein levels were decreased significantly in SLE CD4+ T cells, which are positively correlated with the global DNA methylation levels. Knockdown of BDH2 with siRNA in normal CD4+ T cells decreased the global DNA methylation level compared with negative control. In contrast, overexpressing BDH2 with expression plasmid can increase the global DNA methylation level in SLE CD4+ T cells.
**Conclusions** BDH2 expression was defect in CD4+ T cells of SLE patients, which contributes to the genomic DNA hypomethylation of CD4+ T cells in SLE patients.

## Innate immunity

### 337 THE SIGNIFICANT ROLE OF TOLL-LIKE RECEPTOR IN SYSTEMIC LUPUS ERYTHEMATOSUS

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**Background and aims** Until nowadays, the exact aetiology of SLE (Systemic Lupus Erythematosus) is still unknown. It is currently accepted that there are several factors responsible for complex immunological disorders contributing to its development. Recent studies have shown that abnormal stimulation of innate immunity may have a great influence on the immunopathogenesis of SLE. Since TLRs (Toll-Like Receptors) are essential modulators of innate immune response, its role in SLE pathogenesis has raised great interest, particularly of those recognising nucleic acid, the main antigenic target in SLE.

Analyse the role of TLR in the pathogenesis of SLE.
**Methods** We look up for scienctific article comprehensively in Medline, Science Direct, PubMed, and Cochrane Database. We found 20 article based on bibliography and keywords from the database.
**Results** The expression of TLR7 and TLR9 in peripheral blood mononuclear cells is higher in SLE group compared to control group, while the expression of other TLR shows no difference. There is also a positive correlation between TLR9 expression and activity index and R-SLEDAI score, while TLR7 expression shows positive correlation with chronicity index. Anti-Sm autoantibodies is absent in TLR7 deficiency, while anti-dsDNA autoantibodies are absent in TLR9 deficiency. TLR7 also plays crucial role in B cell proliferation.
**Conclusions** TLR7 and TLR9 appears to play role in pathogenesis of SLE. However, more research need to be done to understand more about the role of TLR in pathogenesis of SLE.

### 338 THE ROLE OF TLR8 IN LUPUS NEPHRITIS

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**Background and aims** Lupus nephritis (LN) is a significant cause of morbidity and mortality. In recent systems biology work we found that renal macrophage functional pathways that link phagocytosis with activation of TLR pathways and disposal of excess cellular components are shared between mice and humans with lupus nephritis. Surprisingly we found that in two different lupus strains there is marked upregulation of renal TLR8 expression restricted to resident renal macrophages.
**Methods** Because mouse TLR8 does not recognise ssRNA, we generated NZW/B6.Yaa mice expressing a functional form of human TLR8 as a BAC transgene to examine the effect of human TLR8 on systemic immunity and renal inflammation. TLR8 mRNA was quantitated by qPCR. Male and female mice were followed clinically for up to one year and harvested for flow cytometry analysis of spleens and kidneys. 24-week-old male mice were administered TL-506 subcutaneously for 4 weeks and harvested after 8 weeks.
**Results** A single dose of human TLR8 in NZW/B6.Yaa mice did not exacerbate disease or accelerate disease or change the immune phenotype of the spleens or kidneys. However administration of a TLR8 agonist to male NZW/B6.Yaa mice appeared to enhance germinal centre formation and plasma cell generation in transgenic mice.
**Conclusions** A single dose of human TLR8 is not sufficient to initiate or exacerbate lupus in NZW/B6.Yaa mice. Preliminary findings suggest that the mice may be hyperresponsive to a TLR8 agonist. Lupus mice with 2 copies of the human TLR8 transgene have been generated to determine whether an extra dose will affect lupus onset or phenotype.

### 339 THE ROLE OF NEUTROPHILS IN ORGAN TISSUE DAMAGE IN SLE

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**Background and aims** Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterised by high levels of autoantibodies and multi-organ damage. Neutrophils are the

most abundant leukocytes in Human blood, but it is not clear whether neutrophils exert an important role in the pathogenesis of organ tissue damage in SLE.
**Methods** We used lupus-prone mouse model and model of lupus serum-induced tissue inflammation in mouse to investigate the role of neutrophils in the organ damage of SLE.
**Results** We found that there was a little neutrophil infiltration in the inflammatory sites of skin, liver, brain and joint in lupus-prone mice. We also found that there was also little neutrophil infiltration in the site of skin inflammation induced by lupus serum in normal mouse. The severity of skin inflammation induced by lupus serum was not significantly decreased in mice with neutrophil depletion compared to ones without neutrophil depletion. But we found that neutrophils were actually involved in tissue injury induced by lupus IgG. Further studies showed that lupus IgG stimulated and activated neutrophils, and cause the death of neutrophils. Studies also confirmed that Fas plays an important role in neutrophils apoptosis.
**Conclusions** Our study indicates that neutrophils participate in the early stage of lupus organ damage, and then they died through activation-mediated apoptosis. These findings promote the understanding of the role of neutrophils in the tissue injury with SLE.

## 340 TLR8 ACTIVATION IN NEUTROPHILS IMPAIRS IMMUNE COMPLEX PHAGOCYTOSIS THROUGH FURIN-DEPENDENT SHEDDING OF FCGRIIA

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**Background and aims** Neutrophils play a crucial role in host defense through mechanisms including phagocytosis and formation of neutrophil extracellular traps (NETs), a recently identified neutrophil cell death process in which DNA is extruded together with cytoplasmic and granular content to trap and eliminate extracellular pathogens. Although beneficial from a host-pathogen perspective, exaggerated neutrophil activation has been linked to autoimmunity, in particular the rheumatic disease systemic lupus erythematosus (SLE) where nucleic acid-containing immune complexes (IC) drive inflammation. Toll-like receptor (TLR) agonists, such as nucleic acids, are important components of pathogens, enabling enhanced phagocytosis by macrophages and dendritic cells, but the role of TLR signalling in processing of SLE ICs and downstream inflammatory neutrophil effector functions is not known.
**Methods** Standard Methods.
**Results** We observed that both FcgR- and TLR8-engagement were required for induction of NETosis, whereas TLR8 activation, through the RNA component of the ICs, suppressed further IC-mediated phagocytosis. Mechanistically, TLR8 ligation induced PI3K-dependent ROS generation through NADPH oxidase, and subsequent furin-dependent proteolytic cleavage of the N-terminal part of FcgRIIA shifting neutrophils away from phagocytosis of ICs toward NETosis. TLR8 activated neutrophils promoted cleavage of FcgRIIA also on plasmacytoid dendritic cells and monocytes resulting in impaired overall clearance of ICs and increased complement C5a generation. Importantly, *ex vivo* derived activated neutrophils from SLE patients demonstrated a similar cleavage of FcgRIIA that was correlated with markers of disease activity as well as complement activation.
**Conclusions** Therapeutic approaches aimed at blocking TLR8 activation would be predicted to increase phagocytosis of circulating ICs while disarming their inflammatory potential.

## 341 CD11B REGULATES INFLAMMATION, AUTOIMMUNITY AND ASSOCIATED PATHOLOGY IN A MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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**Background and aims** Systemic Lupus Erythematosus (SLE) is a highly complex, heterogeneous autoimmune disease characterised by circulating self-reactive antibodies that deposit in tissues including skin, kidneys and brain, alongside a chronic inflammatory response that leads to progressive tissue damage and impaired function. Genome-wide association studies have identified a number of receptors and signal transduction molecules specific for the immune system that predispose to the development of SLE. A loss-of-function single nucleotide polymorphism (SNP) in the *Itgam* gene encoding CD11b (rs1143679) has been identified which associates with an increased incidence of SLE, implicating CD11b as a protective factor against disease development. To understand the role that CD11b plays in controlling autoimmune disease, we crossed CD11b deficient mice ($CD11b^{-/-}$) with Lyn deficient ($Lyn^{-/-}$) mice, a well-studied, robust model of human SLE.
**Methods** Double knockout $Lyn^{-/-}CD11b^{-/-}$ mice were analysed over time for development of autoimmune disease and inflammation.
**Results** While $CD11b^{-/-}$ mice presented with mild splenomegaly and lymphadenopathy, immune cell compartments were unchanged and pathogenic IgG anti-dsDNA autoantibody titres and glomerulonephritis were undetected suggesting that CD11b deficiency alone is insufficient to drive autoimmune disease. Conversely, deficiency of CD11b on the Lyn-deficient autoimmune-prone background exacerbated disease, driving splenomegaly and lymphadenopathy, extramedullary haematopoiesis, autoantibody production and glomerulonephritis, which heavily impacted survival.
**Conclusions** These findings confirm that CD11b is an autoimmune susceptibility gene that when mutated can exacerbate the severity of disease on a susceptible genetic background. This work highlights an important role for CD11b in regulating and controlling the progression of inflammation and autoimmune disease.

## 342 NEW POSSIBILITIES OF HUMAN COMPLEMENT SYSTEM IN DIAGNOSTICS AND ANALYSES OF AUTOIMMUNE DISEASES

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**Background and aims** The aim was to summarise own data on the human complement system involving cofunctioning