

# [Mechanisms of autoimmunity in animal models and humans](https://assignbuster.com/mechanisms-of-autoimmunity-in-animal-models-and-humans/)

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Genetic and cellular mechanisms of autoimmunity in animal models and humans

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| Research Plan.( Do not exceed two pages, including all references, tables and figures. Do not attach separate documents ) Background: Systemic lupus erythematosus (SLE) is a complex genetic trait disease of unknown aetiology characterized by the production of antinuclear antibodies resulting in the formation and deposition of immune complexes that can cause tissue damage (1). Over the past decade genome-wide association studies (GWAS) of lupus-prone murine models, such as New Zealand Black/White (NZB/W) and New Zealand Mixed 2410 (NZM2410) have identified at least 30 lupus-associated loci (2). Despite the technological progress of similar genetic studies in humans, the murine studies remain an invaluable experimental resource to explore the genetic and cellular mechanisms driving the autoimmune response and the tissue injury. Previous studies in the laboratory of my supervisors have shown that the 129xC57BL/6 hybrid strain, a genetic background commonly present in gene-targeted mice, spontaneously develops autoimmunity (3, 4). Subsequent studies identified two 129-derived loci, one on chromosome 1 (named Sle16) and one on chromosome 3 (named Sle18) that, when expressed on a C57BL/6 genetic background, are capable of acting as disease-modifying loci. In particular, it was demonstrated that mice carrying the Sle16 locus (named B6. 129- Sle16 ) spontaneously developed a lupus-like disease and had T cell abnormalities including an enhanced cellular response to anti-CD3 stimulation (5). However, a bicongenic mouse strain with both loci (B6. 129- Sle16Sle18 ) displayed no autoimmune features demonstrating that the Sle18 locus could mitigate the autoimmune features driven by Sle16 (6). The disease-modifying effect of the Sle18 locus was supposed to be mediated, at least in part, by T cells. This hypothesis was based on the observation that CD4 + T from the bicongenic mice ( Sle16Sle18 CD4 + T cells) could dampen the abnormal threshold for proliferation of naive Sle16 CD4 + T, when stimulated with an anti-CD3 antibody (6). Taken together, these findings indicate that the 129-derived Sle18 locus may act as a lupus-suppressive locus by rectifying lupus-related T cell defects. However, the molecular and cellular basis of this disease-modifying effect is still unknown. T cells are known to play a key role in SLE pathogenesis and a wide range of abnormalities has been reported in the literature (7). Recently a study in patients has shown that a specific gene expression profile of CD8+ T cells can be used as biomarker to predict long-term prognosis in two autoimmune diseases: anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and SLE (8). Two distinct CD8+ T cell expression profiles were observed in both conditions. These profiles were found to correlate with disease prognosis. The expression profile defining the poor prognostic group of patients was enriched in genes involved in the regulation of T cell receptor (TCR) and Interleukin-7 receptor (IL-7R) signaling pathways, as well in the expansion of CD8+ memory T cells (8). However, the role of CD8+ T cells in SLE remains poorly understood and whether CD8+ T cells also contribute to the immunomodulatory role of the Sle18 locus has never been explored. Preliminary data: To narrow down the size of the Sle18 locus, my supervisors have generated subcongenic lines (named Sle16. Sle18a and Sle16. Sle18b ). Phenotypic analysis of these shorter overlapping sub-congenic lines confirmed that the region between 71. 5 to 90. 5Mbp (named Sle18a) is responsible for the disease-modifying effects: B6. 129- Sle16Sle18a animals have reduced autoantibody levels, whilst B6. 129- Sle16Sle18b mice display the same autoimmunity phenotype as the lupus-prone strain B6. 129- Sle16 . Therefore, subsequent studies focussed on the genes located in the Sle18a locus. Exome sequencing, carried out in collaboration with the Wellcome Trust Sanger Institute, has revealed that the Sle18a locus contains 187 coding genes, 28 of which contain ≥1 non-synonymous polymorphism between 129 and B6 strains (unpublished data). Since the original data suggested that the disease-suppressive effect of Sle18a is T cell-mediated, to complement the exome sequencing analysis, transcriptomic data from naïve CD4+ T has been recently obtained using RNA-sequencing (Illumina platform) in collaboration with Prof Petretto, Head of Integrative Genomics and Medicine Group, MRC-CSC. This analysis has identified 450 differentially expressed genes in double congenic mice (B6. 129- Sle16Sle18a) compared to C57BL/6 control. Interestingly, juxtaposition of single congenic Sle16 mice to C57BL/6 revealed at least 1700 differentially expressed genes. The reduction of in the number of genes being differentially expressed in the presence of the 129-derived Sle18 locus suggests that a change of regulation in naïve CD4+ T cells may contribute to the lupus resistance phenotype observed (6). How these differentially expressed genes contribute to the suppression of the lupus-like disease remains to be tested. This work is currently in progress in the laboratory of my supervisors and will complement the data obtained during my PhD study.  |

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| 1. In view of the study of McKinney et al. (8), I intend to compare the gene-expression profile of CD8+ T cells from the parental control C57BL/6 strain with that of CD8+ T cells from the different congenic lines (B6. 129- Sle16 , B6. 129- Sle18 and B6. 129- Sle16Sle18a ). This analysis has the potential to unravel the genetic pathways that mediate suppression of autoimmunity in the B6. 129- Sle16Sle18a mice and in long term may have translational benefit for patients with autoimmunity.

Aims and Methods: My PhD project aims to use high-throughput RNA sequencing strategies to investigate the molecular basis of the epistatic interactions between 129-derived Sle16 and Sle18 loci in the development of a lupus-like disease in C57BL/6 mice. The main objectives and methods are as follow: 1. To determine the gene expression profile of CD8+ T cells from lupus-susceptible and lupus-resistant strains using RNA-sequencing (Illumina platform). RNA will be extracted from CD8+ T cells of pre-disease (8-weeks old) congenic mice (B6. 129- Sle16Sle18a, B6. 129- Sle16 and C57BL/6) using T cell isolation kits. Purity of the cell preparations will be confirmed by flow cytometry. Isolated RNA will be subjected to Illumina’s RNA-seq library as per manufacturer recommendation and sequenced. Similarly to the analysis of previous RNA-seq data, the results will be analysed in collaboration with Prof Enrico Petretto, using bioinformatic tools such as fastqc for quality evaluation of raw reads, tophat for alignment of raw reads, Htseq count module to gain counts from alignment, DESeq package for differential expression analysis, DEXsea Bio for alternative splicing analysis and Goseq for gene ontology enrichment analysis. Differences in gene transcripts will be confirmed by quantitative RT-PCR using TaqMan technology. The CD8+ T cell transcriptomic data will complement the genomic data, already available, and the CD4+ T cell transcriptomic data. These datasets will be compared and specific genes of interest will be selected for further studies.
2. To investigate the potential role of shortlisted genes of interest by in silico analysis and functional assays. In order to further elucidate the functions of selected candidate genes, I will use pathway analysis tools such as Generally Applicable Gene-Set Enrichment (GAGE) (9), Gene Set Association Analysis (GSAA) (10) and Ingenuity (11). I will consider using siRNA or RNAi technology to alter gene translation or transcription in T cells to test the cellular effect of these changes. As appropriate, I will develop in vitro and ex vivo T cell functional assays. An example of functional assays that I might use is T cell proliferation assay after stimulation with mitogens (e. g. concanavalin A or phytohemagglutinin, PHA). Flow cytometric assays to assess cell-mediated target cell death and effector-cell frequency/activity will be employed (12).
3. To analyse specific genes of interest inT cells from lupus patients. The murine genomic and transcriptomic datasets will be in silico integrated with human datasets and specific genes of interest will be selected for further studies in humans. The gene expression of selected candidate genes will be analyzed in primary T cells from SLE patients. RNA from CD8+ and CD4+ T cells has already collected in the laboratory of my supervisors. Data will be associated with disease activity score and therapeutic response.

Research Outcome: This research project could potentially reveals novel mechanisms underpinning autoimmunity References: 1. YH Cheung et al. Seminars in immunology . 2009, 21(6): 372-382
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