Mechanisms of autoimmunity in animal models and humans

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Genetic and cellular mechanisms of autoimmunity in animal models and humans

Research Plan.(*Do not exceed two pages, including all references, tables and figure attach separate documents*)

Background:

Systemic lupus erythematosus (SLE) is a complex genetic trait disease of unknown a characterized by the production of antinuclear antibodies resulting in the formation a deposition of immune complexes that can cause tissue damage (1). Over the past de genome-wide association studies (GWAS) of lupus-prone murine models, such as Ne Zealand Black/White (NZB/W) and New Zealand Mixed 2410 (NZM2410) have identifi least 30 lupus-associated loci (2). Despite the technological progress of similar gene studies in humans, the murine studies remain an invaluable experimental resource t explore the genetic and cellular mechanisms driving the autoimmune response and tissue injury. Previous studies in the laboratory of my supervisors have shown that th 129xC57BL/6 hybrid strain, a genetic background commonly present in gene-targete spontaneously develops autoimmunity (3, 4). Subsequent studies identified two 129- loci, one on chromosome 1 (named *Sle16)* and one on chromosome 3 (named *Sle18)* 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 B6. 129- *Sle16*) spontaneously developed a lupus-like disease and had T cell abnorm including an enhanced cellular response to anti-CD3 stimulation (5). However, a bico mouse strain with both loci (B6. 129- *Sle16Sle18*) displayed no autoimmune feature demonstrating that the *Sle18* locus could mitigate the autoimmune features driven b

(6). The disease-modifying effect of the *Sle18* locus was supposed to be mediated, a 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 fo proliferation of naive *Sle16* CD4 $^{+}$ T, when stimulated with an anti-CD3 antibody (6). 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 a 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 abnorm has been reported in the literature (7). Recently a study in patients has shown that a gene expression profile of CD8+ T cells can be used as biomarker to predict long-ter prognosis in two autoimmune diseases: anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) and SLE (8). Two distinct CD8+ T cell expression profiles observed in both conditions. These profiles were found to correlate with disease prog The expression profile defining the poor prognostic group of patients was enriched in involved in the regulation of T cell receptor (TCR) and Interleukin-7 receptor (IL-7R) s pathways, as well in the expansion of CD8+ memory T cells (8). However, the role of cells in SLE remains poorly understood and whether CD8+ T cells also contribute to t 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 subcong 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 *Sle18a)* is responsible for the disease-modifying effects: B6. 129- *Sle16Sle18a* anima reduced autoantibody levels, whilst B6. 129- *Sle16Sle18b* mice display the same

autoimmunity phenotype as the lupus-prone strain B6. 129- *Sle16* . Therefore, subse
studies focussed on the genes located in the *Sle18a* locus. Exome sequencing, carrie
collaboration with the Wellcome Trust Sanger Institute, has revealed that the *Sle18a*
contains 187 coding genes, 28 of which contain ≥1 non-synonymous polymorphism
129 and B6 strains (unpublished data). Since the original data suggested that the dis
suppressive effect of *Sle18a* is T cell-mediated, to complement the exome sequencin
analysis, transcriptomic data from naïve CD4+ T has been recently obtained using R
sequencing (Illumina platform) in collaboration with Prof Petretto, Head of Integrative
Genomics and Medicine Group, MRC-CSC. This analysis has identified 450 differentia
expressed genes in double congenic mice (B6. 129- *Sle16Sle18a)* compared to C57B
control. Interestingly, juxtaposition of single congenic *Sle16* mice to C57BL/6 reveale
least 1700 differentially expressed genes. The reduction of in the number of genes b
differentially expressed in the presence of the 129-derived *Sle18* locus suggests that
change of regulation in naïve CD4+ T cells may contribute to the lupus resistance ph
observed (6). How these differentially expressed genes contribute to the suppression
lupus-like disease remains to be tested. This work is currently in progress in the labo
my supervisors and will complement the data obtained during my PhD study.

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

Aims and Methods:

My PhD project aims to use high-throughput RNA sequencing strategies to investigat molecular basis of the epistatic interactions between 129-derived *Sle16* and *Sle18* lo development of a lupus-like disease in C57BL/6 mice. The main objectives and meth as follow:

2. To determine the gene expression profile of CD8+ T cells from lupus-susceptib lupus-resistant strains using RNA-sequencing (Illumina platform). RNA will be e from CD8+ T cells of pre-disease (8-weeks old) congenic mice (B6. 129- *Sle16* B6. 129- *Sle16* and C57BL/6) using T cell isolation kits. Purity of the cell prepar will be confirmed by flow cytometry. Isolated RNA will be subjected to Illumina seq library as per manufacturer recommendation and sequenced. Similarly to t analysis of previous RNA-seq data, the results will be analysed in collaboration Prof Enrico Petretto, using bioinformatic tools such as fastqc for quality evaluat raw reads, tophat for alignment of raw reads, Htseq count module to gain coun 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 technology. The CD8+ T cell transcriptomic data will complement the genomic already available, and the CD4+ T cell transcriptomic data. These datasets will compared and specific genes of interest will be selected for further studies.
3. To investigate the potential role of shortlisted genes of interest by *in silico* ana functional assays. In order to further elucidate the functions of selected candid genes, I will use pathway analysis tools such as Generally Applicable Gene-Set Enrichment (GAGE) (9), Gene Set Association Analysis (GSAA) (10) and Ingenui will consider using siRNA or RNAi technology to alter gene translation or transc in T cells to test the cellular effect of these changes. As appropriate, I will deve

vitro and *ex vivo* T cell functional assays. An example of functional assays that use is T cell proliferation assay after stimulation with mitogens (e. g. concanav phytohemagglutinin, PHA). Flow cytometric assays to assess cell-mediated tar death and effector-cell frequency/activity will be employed (12).

4. To analyse specific genes of interest inT cells from lupus patients. The murine and transcriptomic datasets will be *in silico* integrated with human datasets an specific genes of interest will be selected for further studies in humans. The ge expression of selected candidate genes will be analyzed in primary T cells from patients. RNA from CD8+ and CD4+ T cells has already collected in the labora my supervisors. Data will be associated with disease activity score and therape response.

Research Outcome:

This research project could potentially reveals novel mechanisms underpinning autoimmunity

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