Genetic Basis of
Systemic Lupus Erythematosus

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- The report of spontaneous autoimmune hemolytic anemia in the NZB/B1 strain 50 years ago not only provided the first example of a lupus-related manifestation in mice but also supported the possibility of this autoimmune disease being an inherited trait.

- Genetic variation was first shown to be important in human SLE in the 1970s with associations in the human leukocyte antigen region.
Spontaneous and induced mouse models of lupus:

**Spontaneous Disease Models**

Hypergammaglobulinemia, ANA (+), glomerulonephritis (GN)

- NZ and related strains
  * Most common: (NZBxNZW) F1
  * Hemolytic anemia (HA)
- MRL-Fas\textsuperscript{\textit{Ipr}} and related strains
  * Arthritis, ↑ DN T cells
- BXSB and related strains
  * Monocytosis

**Induced Disease Models**

- Heavy metal-induced autoimmunity
- Drug-induced autoimmunity
- Pristane (TMPD)-induced
- Anti-idiotypic
- Graft-versus-host disease
- BCG-injected NOD
- Bovine thrombin - exposed galactose-alpha1-3-galactose-deficient mice

***Lupus-like manifestations in SV129xB6 mixed lines.***
**Genetic Factors**

- 10-20% of SLE patients have an affected first-degree relative

- 1/3 concordance in monozygotic twins

- Candidate gene approach, family-based linkage studies, genome-wide association studies (GWAS)

- Single genes (Complement - C2, C4, C1q) - occasional patients

- Multiple loci, genes (100 or more genes)

- SNPs in many genes

- The number of genes involved in expression of autoimmune disease may vary in number; fewer for organ specific and more for the systemic autoimmune diseases.
SLE associated loci and genes in human genome
Innate Immunity

Mouse genetics

- Overexpression of IFN-γ in suprabasal layer of epidermis

- TTP-/- (Tristetrapolin): TTP destabilizes TNF-α mRNA.

- TNF receptors-/-

** Physiological levels of TNF-α suppresses autoimmunity.
**Innate Immunity**

**Human genetics**

- **IFN signature** in SLE patients: increased levels of IFN-inducible genes, correlation with more severe disease manifestations.

**TLR/IFN signaling (Dendritic cells, macrophages):**

- **IRF5** (Interferon Regulatory Factor 5): regulates type 1 IFN-responsive genes, positive feedback loop with IFN-α; multiple susceptible polymorphisms resulting in increased stability; suggesting the model of TLR 7/9 signaling triggered by nucleic acid containing autoantibody complexes or necrotic cell debris.

- **IRAK1** (IL-1 receptor associated kinase) links several immune receptor complexes to central adaptor/activator protein TRAF6, also in T cell signaling.
- **STAT4**: signaling through IFN-α, IL-12 and IL-23; SNPs; originally identified in RA, also in T cell signaling.

- **SPP1** (osteopontin): critical for IFN-α production in pDC; overexpressed in SLE patients, associated with early onset disease.

- **TREX1**: 3’ DNA repair exonuclease 1; potent type 1 IFN response.

**TNF/NFκB signaling (Dendritic cells, macrophages):**

- **TNFAIP3** (Tumor necrosis factor-α-induced-protein 3/zinc finger A20 protein): ubiquitination of molecules activated by TLRs, IL-1R, TNFR, NOD2.
Complement and immune complex clearance
- Macrophages & Neutrophils

Mouse genetics

- $C1q^{-/-}$ / $C4^{-/-}$ on MRL-Fas$^{lpr}$ background

- $DNase1^{-/-}$: Major nuclease in blood, urine, secretions.

  ** Identical heterozygous nonsense mutation in $DNASE1$ in 2 SLE patients, reduced $DNase1$ activity in sera of SLE patients.

- $MFG-E8^{-/-}$ (Milk fat globule-EGF factor 8): MFG-E8 promotes apoptotic cell engulfment.

- $TAM^{-/-}$ (Tyro3, Axl, Mer): TAM promotes clearance of apoptotic cells and inhibit inflammation.

- $Trex1^{-/-}$ (DNA exonuclease): myocarditis & antibodies to heart tissue

- $Ro^{-/-}$: Ro is the common target for autoantibodies in SLE, Sjogren’s Syndrome, neonatal lupus, primary biliary cirrhosis.
Complement and immune complex clearance
- Macrophages & Neutrophils

Human genetics

- Complement components and effective immune complex clearance are important in development of SLE and serious manifestations such as nephritis.

** CNV (Copy number variants) are polymorphisms that arise when an entire gene or gene segment is duplicated or absent in some individuals.

- **C2, C4A, C4B** and **C1q**: levels have strong associations with SLE; decreased copy numbers: high risk for SLE, increased copy numbers: protective against SLE.

* 75% of individuals who are null at the **C4** locus develop SLE.
The short arm of chromosome 6

Genetic susceptibility

DP  DQ  DR  C4  C2  TNF  B  C  A

class I

C1q
- **FCGR3A and FCGR2A**: activating receptors and members of the immunoglobulin superfamily; FcγRIIA on NK cells, macrophages and some monocytes & FcγRIIIA on neutrophils; missense mutations alter IgG binding and immune complex processing.

- **CRP** (C reactive protein): binding to FcγRI or FcγRIIA leads to phagocytosis and release of inflammatory cytokines; binding to FcγRIIB blocks its activating signal. Genetic variations in the promoter and CNV determines CRP levels which is highly associated with SLE.

Antigen Presentation - MHC

Mouse genetics

- Overexpression of CD40L on basal keratinocytes: activation of resident tissue APCs (Langerhans cells)

- Dcir⁻/⁻ (Clec4a2): excess expansion of DCs and enhanced antigen presentation.
Antigen Presentation - MHC

Human genetics

- 120 genes in the HLA (Human Leukocyte Antigen) Class I, II and III regions are important in immune function.

- Class II HLA-DR and HLA-DQ molecules

- Class III HLA genes: more important susceptibility genes for SLE.

  **MSH5 (mutS homologue 5) gene:** DNA rearrangements that lead to immunoglobulin class switching.

  **SKIV2L (super viralicidic activity 2-like) gene:** RNA helicase, highly expressed in T,B and dendritic cells.
TCR

T cell mediated lupus phenotype

LAT
Cbl, Cbl-b
G2A
E2F2
Shc1
Dok1, Dok2
Mgat5
Gadd45
Def6

Ligand/Receptor Interactions:
- TCR
- LIGHT
- CTLA-4
- PD-1
- PDL-1
- CD28
- B7.1-B7.2
- TGF-β
- TGF-β receptor
- ICOS
- Roquin

B cell

TGF-β
**Lymphocyte Signaling - T cells**

**Mouse genetics**

- **TGF-β1**<sup>−/−</sup> - massive disease, death at 3 weeks
- **CTLA-4**<sup>−/−</sup> - multiorgan lymphoproliferative disease, death at 3-4 weeks
- **PD1**<sup>−/−</sup>/**PDL1**<sup>−/−</sup> - strain dependent phenotype. PD1 inhibits TCR-mediated proliferation and cytokine secretion.

- Overexpression of **LIGHT/Tnfsf14** - costimulatory molecule for T cell activation
- **LAT loss-of-function mutation** - severe block in TCR signaling and T cell development
- **Cbl-b**<sup>−/−</sup> / **Cbl&Cbl-b DKO** in B cells - enhanced proliferation to antigen receptor signaling, impaired anergy to self-antigen
- **G2A**<sup>−/−</sup> - G2A negatively regulates proliferation and to integrate of extracellular signals with cytoskeletal organization.

- **E2F2**<sup>−/−</sup> - lowers TCR activation threshold and more rapid entry of activated T cells into S phase.
- **Shc1** (p46, p52, p66), **p66**<sup>−/−</sup> - p66 suppresses antigen-receptor signaling in both T and B lymphocytes.
- **Dok1&Dok2 DKO** - Dok1&Dok2 suppress TCR signaling.
- **Roquin** (Rc3h1) loss-of-function mutation - Roquin regulates mRNA translation and stability, repressor of ICOS.

- **Mgat5**<sup>−/−</sup> - enhanced TLR clustering and signaling
- **PCMT**<sup>−/−</sup> - hyperresponsive T cells
- **Gadd45a**<sup>−/−</sup> / **Gadd45β**<sup>−/−</sup> - enhanced T cell activation
- **Def6** (IRF4 binding protein)<sup>−/−</sup> - decreased TCR signaling
Lymphocyte Signaling - T cells

Human genetics

- Reduced TCR (T cell receptor) signaling increases risk for autoimmunity.

- **PTPN22/LYP** (protein tyrosine phosphatase 22 - lymphoid-specific tyrosine phosphatase): polymorphisms increasing phosphatase/catalytic activity, originally identified as a candidate gene for type 1 diabetes and RA; more robust in familial SLE.

- **TNFSF4/OX40L** on antigen presenting cells-TNFSFR4/OX40 on activated T cells: costimulatory pathway, destabilizing peripheral tolerance, increased expression of TNFSF4.

- **PDCD1** (programmed cell death 1 gene): immunoinhibitory receptor belonging to B7/CD28 family; intronic SNP disrupting the promoter activation → lowering the threshold for response to self-antigen.
B cell mediated lupus phenotype

- BCR
- CD40
- CD40L
- Act-1
- BAFF-R
- TACI
- BAFF
- Aiolos
- miR-17-92
- SLC
- PKC-δ
- PLCγ2
- Rai
- SPA-1
- PECAM-1
Lymphocyte Signaling - B cells

Mouse genetics

- Overexpression of BAFF/BLyS/Tnfsf13b
- TACI (Tnfrsf13b)^/-
- Aiolos^/- (zinc finger transcription factor) - increased response to BCR and CD40.
- Ectopic CD40L expression on B cells
- Act1^/- (CISK) - Act1 negatively regulates BAFF and CD40L.
- SLC^/- (surrogate light chain)
- PKC-δ^/- - PKC-δ plays role in tolerogenic, not immunogenic BCR signaling.
- PL (phospholipase) Cγ2 gain of function mutation - PLCγ2 catalyzes phosphoinositides to diacylglycerol and inositol phosphates.
- Overexpression of Fli-1 - Ets transcription factor family member
- Rai (Shc3)^/- - Rai negatively regulates BCR signaling and cel activation. Inefficient receptor editing in B cells.
- SPA1^/- - SPA1 inhibits Rap1 in controlling cell adhesion and MAP kinases.
- PECAM-1 (CD31)^/- - Immunoglobulin superfamily
- Overexpression of miR-17-92 cluster
Lymphocyte Signaling - B cells

Human genetics

- **BANK1** (B cell scaffold protein with ankyrin repeats): B cell adaptor protein, 3 variants increasing lupus.

- **LYN**: protein tyrosine kinase, associates with BCR and BANK1.

- **BLK (B lymphoid tyrosine kinase)**: member of Src family, influence cell proliferation, differentiation and B cell tolerance; reduced expression of BLK mRNA in SLE; promoter shared with C8orf13.
Chromosome X

Human genetics

- Women are affected 10-fold more than men. Direct effects of sex chromosomes or indirect effects of sex hormones? Epigenetic changes on X chromosome? Gene dose effect?

  - **IRAK1**: gene dosage

  - **MECP2** (methyl-CpG-binding protein 2 gene): potential role for DNA methylation/demethylation in the pathogenesis of SLE. Genes that were upregulated had significantly more CpG (demethylated) islands in their promoter regions.

  - **CD40L**: demethylation of the gene on inactive X may predispose women to SLE due to CD40L overexpression.
**Unknown function**

Human genetics

- **KIAA1542**: genetic effect due to close proximity to IRF7 gene?

- **PXK, XKR6, FAM167A**: no known relation to immune function, lupus pathogenesis and autoimmunity.
Gene-Gene and Gene-Environment Interactions

Human genetics

- IRF5 - TYK2: OR=2.73, additive interaction
- IRF5 - STAT4: additive interaction
- NAT2 slow acetylator genotype - smoking: OR=6.44, additive interaction
Conclusions:

- Genetic variation is the basic determinant of host contribution to phenotype.

- Genes and loci which are involved in SLE phenotype are widely distributed throughout the genome.

- Studies to identify and characterize the genes required for the development of SLE have relevance for both basic understanding of disease process and for therapy.