



Review

Emerging Concepts in the Molecular Pathogenesis of Systemic Lupus Erythematosus

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Abstract. Systemic lupus erythematosus is a prototypic autoimmune disease that afflicts predominantly women during their child-bearing age. The disease is characterized by the production of autoantibodies and immune complexes in association with a diverse array of clinical manifestations. Investigation into the etiopathogenesis has been directed at identifying the genes that provide susceptibility to the disease, the complex cellular and cytokine aberrations and the biochemical abnormalities that are responsible for them. Understanding the immune cell signaling and gene transcription abnormalities will help us tailor new strategies for efficient biotherapy of the disease.

Key words: systemic lupus erythematosus; autoimmune diseases; pathogenesis.

Introduction

Systemic lupus erythematosus (SLE) is an idiopathic autoimmune disease characterized by disorders of cellular and humoral immune response leading to autoantibody production. SLE predominantly afflicts woman (9:1 compared with men) in their child-bearing age. People of African, Afro-Caribbean, Asian, Native American and East Asian descent are more likely to develop SLE and may have a more severe course of disease and prognosis than Caucasians. The autoimmune response leads to abnormal production of a wide spectrum of autoantibodies forming immune complexes that deposit on tissues propagating a chronic inflammatory process that destroys organ parenchyma and results in end-stage organ failure. The precise pathogenic tissue bound antibody, immune complexes, autoreactive T cells and by-products of the immune cell activa-

tion, like cytokines, are the principal effectors of this inflammatory process. The current treatment of SLE centers on regimens that include corticosteroids and cytotoxic drugs.

Despite considerable research, the etiology of SLE remains elusive. Genetic, hormonal, environmental and immunomodulatory factors contribute to the pathogenesis of SLE. A central role for T cells is suggested in the pathogenesis of the disease, and immune aberrations of SLE T cells are considered to be the primary event in the pathologic process. Identifying the underlying genetic and biochemical mechanisms will contribute to our understanding of the etiopathogenesis of SLE and identify novel targets for pharmacological intervention. Here we discuss the newest developments in the molecular and cellular pathogenesis of human lupus with more emphasis on abnormal immune cell signaling.

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Etiologic Factors Involved in Human SLE

Genome-wide searches in families with multiple affected members revealed the involvement of a large number of genes contributing to the expression of the disease and are distributed through out the genome, although most cluster in the 1q and 6p chromosomes^{10, 13, 53} (Table 1). The latter includes genes for HLA and complement factors. Etiologic genes in these disorders determine susceptibility, and no particular gene is necessary or sufficient for disease expression. However, almost all of the patients with deficient C1r/C1s C2 and C4 genes that are integral to the immune response develop SLE. In African Americans, chromosome region 1q was found to have the strongest linkage and candidate genes in this interval include FcγRIIA, FcγRIIIB and the ζ chain of the T cell receptor (TCR) complex¹⁶. Environmental factors include ultraviolet light, a large list of medications, and infections including viruses and other pathogens. Predominant female preponderance of SLE suggests that hormonal factors play a critical role in SLE pathogenesis. Hormonal factors (estrogens) are very important and are involved in the regulation of the transcription of genes central to the expression of SLE. In T cells of females with SLE, estradiol augments expression and activity of calcineurin suggesting that there is a gender-specific estrogen receptor dysfunction that might modulate immune response and contribute the autoimmune diathesis⁴². No single factor can cause the disease; multiple factors should act simultaneously or sequentially to cause the disease. Relative contribution of each factor varies among patients. A summary of the proposed view of pathogenesis of SLE is shown in Fig. 1.

Table 1. Possible susceptibility loci for systemic lupus erythematosus

Complement pathway	
C2, C4, C1q	
complement receptor 1 and 2	
mannose binding protein	
Human major histocompatibility complex	
DR3, DR2, DQ2, DQ6 containing haplotypes	
Lymphokines	
tissue necrosis factor α (TNF-α gene promoter)	
TNF receptor,	
IL-6, IL-10	
Fc receptors	
FcγRIIA, FcγRIIIB	
Apoptosis factors	
Poly (ADP-ribose) polymerase	
Bcl2	
Fas ligand	
Cell signaling	
PKA-I and PKA-II	

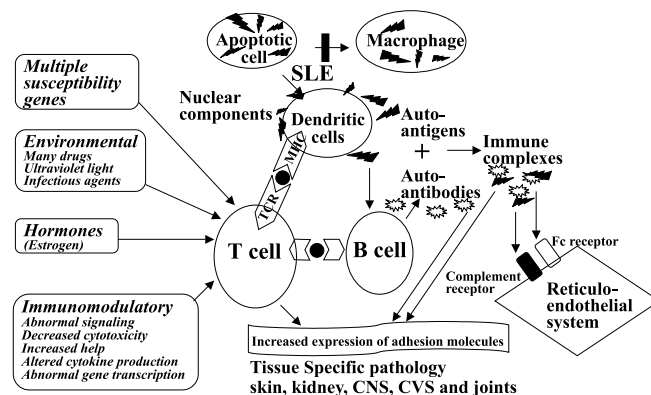


Fig. 1. Schematic view of the summary of etiopathogenic processes in systematic lupus erythematosus. In SLE, apoptotic cells are not properly cleared by macrophages, exposing antigen-presenting cells and B cells to nuclear components of the apoptotic cells. Antigen presentation occurs resulting in the production of autoantibodies that interact with autoantigen forming immune complexes. Decreased rate of the clearance of the autoantibodies and immune complex by the reticuloendothelial system through the Fc and complement receptor leads to its deposition in various tissues cause diverse clinical manifestations. In addition, activated cells may home inappropriately in tissues developing vasculitis. Multiple etiologic factors (shown in italics) contribute to the pathogenesis of SLE and relative contribution of each factor varies among individuals

Immune Cell Aberrations

Immune system abnormalities seen in SLE are multiple and diverse^{54, 55}. Central among them is the presence of overactive B cells responsible for the production of autoantibodies. B cells operate under the control of T cells, which provide cognate help by engaging costimulatory pairs of molecules on the surface of interacting immune cells. Accumulating evidence suggests that a fundamental disorder of both CD4⁺ T helper and CD8⁺ T cell cytotoxic/suppressor functions exists in SLE⁵⁴. The physiologic ratio of circulating CD4⁺ Th1 to Th2 is skewed toward Th2 cells in SLE, resulting in a diminished generation of interleukin 2 (IL-2), and interferon γ (IFN-γ) by Th1 cells and augmented production of IL-6 and IL-10¹⁵. Activated CD4⁺ Th cells bearing increased cell surface CD40 ligand (CD40L) and CD11a/CD18 (lymphocyte function associated antigen-1) overproduce IL-6 and IL-10. In turn, CD4⁺ Th2 cells interact with B cells driving overproduction of immunoglobulins by pathogenic B cell clones directed against components of cell nucleus in human SLE. Simultaneously, in response to antigens such as tetanus toxoid and viruses, the T cells display decreased cellular cytotoxic responses and decreased production of IL-2 that may contribute to the increased rate of infections in SLE patients.

T Cell Signaling Abnormalities

Immune cells respond to external antigens following their engagement to the antigen receptor through a series of biochemical processes involving protein tyrosine phosphorylation, calcium mobilization and activation of transcription factors, known as cell signaling. The antigen receptor, TCR/CD3, is a multisubunit complex consisting of α and β chains, the CD3 γ , δ , and ϵ chains, and a ζ - ζ homodimer^{60, 61} or heterodimer of ζ with η chain or γ chain of the high affinity IgE receptor Fc ϵ RI. The disulfide-linked α - β heterodimer is responsible for antigen recognition, whereas CD3 ϵ - δ , CD3 ϵ - γ , and ζ - ζ homodimers couple with the α - β chains to initiate intracellular signal transduction. Each subunit of the ζ chain homodimer possesses three immune receptor tyrosine-activated motifs (ITAMs) whereas other CD3 chains contain one ITAM each. Upon TCR activation, the tyrosine residues within the ITAMs become phosphorylated by Lck and Fyn, leading to the association and activation of ZAP-70. Once activated, Fyn, Lck, Syk and ZAP-70 cooperate in the tyrosine phosphorylation, activation and juxtaposition

of downstream signal transducers that contribute to the initiation of microtubule-associated protein (MAP) kinase cascades, PI3-kinase activation, Ca^{2+} flux, and activation of transcription factors (Fig. 2). Eventually, these transcription factors translocate to the nucleus and modulate the expression of genes that regulate lymphocyte activation, cellular proliferation, anergy or apoptosis, secretion of soluble mediators and effector functions.

Following engagement of the antigen receptor, T and B lymphocytes from SLE patients respond rapidly by hyperphosphorylating a number of cytosolic signaling protein intermediates and increasing their concentration of free calcium^{31, 59} (Table 2). This abnormal response occurs in both principal T cell subsets and in cell cultures propagated *in vitro*. In SLE T cells, the more sustained supranormal calcium response has a faster kinetics and is observed under deficient TCR ζ chain expression^{3, 30, 51}. The deficient TCR ζ chain has also been associated with decreased activation induced cell death in SLE T cells²⁷. The TCR ζ chain is considered to be the limiting factor in T cell receptor assembly, transport and surface expression and is cru-

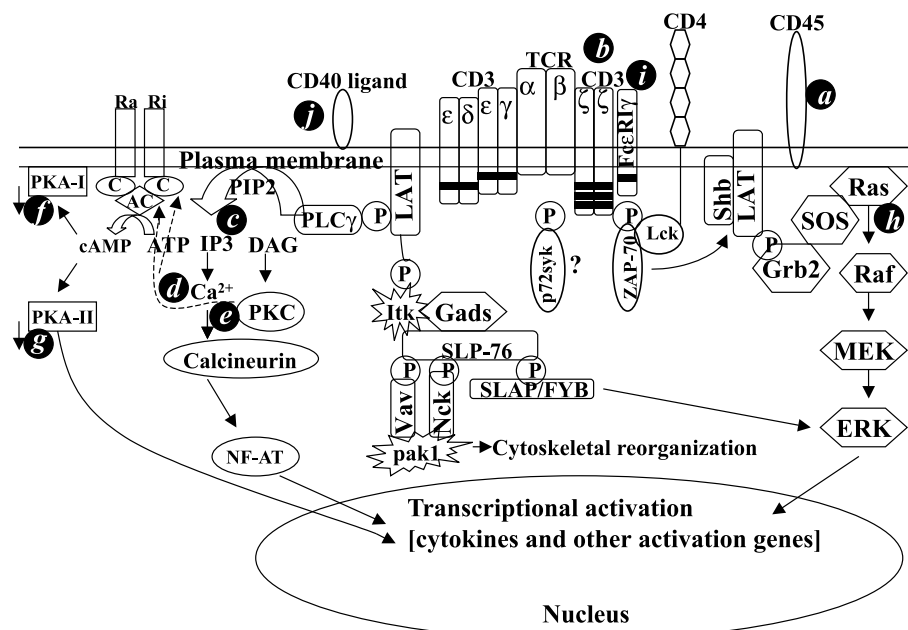


Fig. 2. The current model of T cell signal transduction in T lymphocytes depicting the molecular defects identified in SLE. Upon TCR activation by antigen, the tyrosine residues within the three ITAMs (—) become phosphorylated by Lck and Fyn, leading to the association and activation of ZAP-70. Once activated, Fyn, Lck, Syk and ZAP-70 cooperate in the tyrosine phosphorylation, activation and juxtaposition of downstream signal transducers that contribute to the initiation of MAP kinase cascades, PI 3-kinase activation, Ca^{2+} flux and activation of transcription factors. Eventually these transcription factors translocate to the nucleus and modulate the expression of genes that regulate lymphocyte activation, cellular proliferation, anergy or apoptosis, secretion of soluble mediators and effector functions. Signaling abnormalities currently identified in SLE T cells are indicated in black circles. a – deficient CD45 phosphatase; b – TCR ζ chain deficiency; c – moderate increase in inositol trisphosphate (IP3); d – increased and sustained intracellular calcium levels; e – reduced PKC phosphorylation; f – deficient protein kinase A I activity; g – impaired protein kinase A II activity and increased nuclear translocation of the free RII β subunit; h – reduced Ras-MAP kinase signaling; i – increased expression of Fc ϵ RI γ chain that replaces the deficient TCR ζ chain; j – increased expression of CD40 ligand

Table 2. Molecular defects of the T cell signaling pathways in SLE

Molecular defect	Functional effect	Reference
CD45 tyrosyl phosphatase	deficient	50
TCR ζ chain	deficient, increased lipid raft association	3, 30, 51
Fc ϵ RI γ	increased	9
Lck tyrosyl kinase	deficient	33
Inositor triphosphate	increased	59
Calcium	increased and more sustained	59
PKA-I and PKA-II isozymes	deficiencies	21, 34
Protein kinase C	deficiency	49
NF- κ B, p65 Rel A subunit	deficiency	64
Elf-1	deficiency	(JUANG et al., unpublished data)
p-CREM	upregulation and increased nuclear binding to -180 site of IL-2 promoter	48
Ras-MAP kinase	deficiency	8
CD40L	increased	25

cial to receptor signaling function^{60, 61}. TCR ζ chain also has been implicated in the selection of the TCR repertoire and in the prevention of autoimmunity. A vast majority of the SLE patients also display a decreased expression of TCR ζ chain mRNA. The TCR ζ chain transcript is generated as the spliced product of 8 exons that are separated by distances of 0.7 kb to more than 8 kb¹⁹. TCR ζ chain gene is located in chromosome 1q23^{47, 62}. Genes encoded in chromosome 1q have been suggested to contribute to genetic predisposition and susceptibility to SLE by genome-wide scans of multiplex SLE families^{10, 11, 13, 36, 44}. Genetic linkage of the TCR ζ chain gene to the Fc γ RII and F γ RIII gene cluster, a candidate locus implicated in genetic susceptibility to SLE, suggest that TCR ζ chain might play an important role in genetic predisposition to SLE¹⁹.

Although the precise molecular mechanisms underlying ζ chain deficiency is still being examined, current evidence supports the possibility of a transcriptional defect. In SLE T cells that expressed low levels of TCR ζ chain transcripts, cloning and sequencing revealed more frequent heterogeneous polymorphisms/mutations and alternative splicing of TCR ζ chain^{38, 51, 58}. Most of these mutations are localized to the three ITAMs or guanosine triphosphate (GTP)-binding domain and could functionally affect the ζ chain providing a molecular basis to the known T cell signaling abnormalities in SLE T cells. Absence of the mutations/polymorphisms in the genomic DNA suggests that these are the consequence of irregular RNA editing. SLE patients also showed significant increase in the splice variants of the ζ chain. The splicing abnormality included two insertion splice variants of 145 bases and 93 bases between exons I and II and also several deletion splice variants of TCR ζ chain resulting from the deletion of individual exons II, VI, VII or a combined deletion of

exons V and VI; VI and VII; II, III and IV; and V, VI and VII in SLE T cells³⁷. Transfection of a mouse ζ -deficient cell line, MA5.8, with the alternatively spliced isoforms of the TCR ζ chain and TCR/CD3 activation, showed functional variation among the splice variants and the wild-type TCR ζ chain. However, the surface expression of the TCR remained similar in MA5.8 cells transfected with the alternatively spliced isoforms of the TCR ζ chain, suggesting that the splice variation does not impair assembly, transport and surface expression of TCR.

Real time PCR analysis of the TCR ζ chain 3'untranslated region showed an alternatively spliced 344 bp product with both splicing donor and acceptor sites, resulting from deletion of nucleotides from 672 to 1233 of TCR ζ chain mRNA. Unlike the normal TCR ζ chain, the expression of the TCR ζ chain with the alternatively spliced 344 bp 3'untranslated region was higher in SLE T cells compared to non-SLE controls. Preliminary studies show that the stability of the TCR ζ chain with the alternatively spliced 3'untranslated region is more unstable leading to its downregulation in SLE T cells (Tsokos et al., unpublished data). Since, many of the principal antinuclear antibodies in SLE are directed against spliceosome components, it is not clear whether these autoantibodies play any role in the defective mRNA synthesis, splicing and processing in SLE T cells.

Abnormalities of Elf-1 Expression

Analysis of the TCR ζ chain transcription factor Elf-1 indicates that the p98 form of the protein was decreased in the majority of the lupus patients, whereas

p80 form was increased. Elf-1 is a member of the E twenty-six specific (Ets) transcription factor family. Elf-1 has a calculated molecular weight of 68 kDa while we found that it existed in T cells as both 80 and 98 kDa forms. We determined that the 80 kDa Elf-1 was mainly located in the cytoplasm while the 98 kDa Elf-1 was the major nuclear form. Consistently, immunoprecipitation disclosed that retinoblastoma protein (Rb), which retained Elf-1 in the cytoplasm, displayed higher affinity for the 80 than the 98 kDa form, indicating that the conversion from 80 to 98 kDa form allows the Elf-1 to evade the tethering of Rb and facilitate its nuclear translocation. We found the molecular basis for this conversion involving dual post-translational processes, i.e. glycosylation and phosphorylation. Specifically, N-acetylglucosaminidase, but not the endoglycosidase H, digested efficiently the 98 kDa Elf-1, whereas actin was not affected. The 98 kDa form was further resolved into three bands and all bands were either enhanced or suppressed following treatment with okadaic acid or bacteriophage λ phosphatase, respectively. In contrast, only a minor portion of the 80 kDa Elf-1 was in the phosphorylated state subjected to the similar regulation by okadaic acid and phosphatase as the 98 kDa form, indicating that the 98 kDa Elf-1 is the active form. Electrophoretic mobility shift assays and ultraviolet crosslinking studies indicated that the phosphorylated 98 kDa Elf-1 is the functional form that binds to the TCR ζ chain promoter. Nuclear proteins from approximately 40% of SLE T cells displayed decreased production of the 98 but not the 80 kDa form, which correlates well with their defective TCR ζ chain promoter binding. Another 20% of SLE T cell nuclear proteins are defective in TCR ζ chain promoter binding despite normal expression of both the 98 and 80 forms of Elf-1. The defective formation of functional 98 kDa Elf-1 thus underlies the defective TCR ζ chain expression in SLE patients (JUANG et al., manuscript submitted).

The TCR ζ chain exists in multiple forms and membrane fractions with distinct function in the Ag-mediated signaling process. Studies on the complete spectrum of expression of various molecular forms of the TCR ζ chain has shown that the phosphorylated 21 and 23 kDa forms of the TCR ζ chain are significantly decreased in SLE T cells compared to normal T cells. In contrast, major ubiquitinated forms of the TCR ζ chain were increased in SLE T cells, suggesting that TCR ζ chain undergoes an enhanced ubiquitin-mediated degradation in SLE T cells. The level of TCR ζ chain was also significantly decreased in the detergent-insoluble membranes in SLE T cells. Similarly, the expression of

TCR η chain, an alternatively spliced form of the ζ chain, was diminished in SLE T cells. Recently, we identified upregulation of a novel 14 kDa form of the ζ chain, a potential alternatively spliced or degraded species of the TCR ζ chain in SLE T cells (NAMBIAR et al., Arthritis Rheum., in press, Jan. 2002).

Mechanisms of Supranormal TCR/CD3-Mediated $[Ca^{2+}]_i$ Response in SLE

How does the TCR engagement induce hyperphosphorylation of cytosolic proteins and supranormal $[Ca^{2+}]_i$ response in the milieu of deficient TCR ζ chain has been a topic of intense research for the last couple of years. In addition to the possible gain-of-function mutations of the TCR ζ chain, our investigation has proposed two mechanisms, involving increased expression of Fc ϵ RI γ chain and increased membrane lipid-raft association of the residual TCR ζ chain, that could explain the supranormal TCR/CD-mediated $[Ca^{2+}]_i$ response in SLE T cells. Also, the protein tyrosine phosphatase activity of CD45 on peripheral blood lymphocytes is reduced in SLE⁵⁰.

Fc ϵ RI γ Chain Replace the Defective TCR ζ Chain

The hypothesis that, other members of the ζ chain family may substitute for the deficient TCR ζ chain, was first investigated by ENYEDY et al.⁹. Immunoprecipitation/immunoblotting and confocal microscopy demonstrate that a large proportion of SLE T cells express very high levels of Fc ϵ RI γ that is functionally associated with the TCR and takes part in antigen receptor mediated signal transduction. Expression of Fc ϵ RI γ , *in lieu* of the TCR ζ chain, has been reported in mouse large granular lymphocytes⁴³. T lymphocytes from tumor-bearing mice expressed TCR that completely lacked the TCR ζ chain was replaced by Fc ϵ RI γ chain¹. Also, TCR ζ -deficient mice have been shown to express Fc ϵ RI γ as part of the TCR- $\gamma\delta$ complex^{24, 52, 63}. Unlike the TCR ζ chain, which mediates signaling through ZAP-70, Fc ϵ RI γ mediates signaling by associating with the 100-fold more potent phosphorylated protein kinase Syk^{2, 6}. Presently it is unknown, whether Syk is upregulated in SLE T cells and Fc ϵ RI γ signal transduce by associating with Syk or other downstream signaling molecules. High-level expressions of Syk and alternative antigen receptor mediated signaling has been described in T cells of patients with ZAP-70 defi-

ciency³⁹. Overexpression of FcεRIγ in normal T cells also show increased TCR/CD3-mediated $[Ca^{2+}]_i$ response suggesting that the single ITAMs in the FcεRIγ, compared to the three in TCR ζ chain, does not hinder the level of $[Ca^{2+}]_i$ response (KUMAR et al., unpublished results). The high level expression of FcεRIγ chain could replace the defective TCR ζ chain and contribute to the aberrant antigen receptor-initiated signaling in SLE T cells.

Increased Membrane Lipid-Raft Association of the Residual TCR ζ Chain

The TCR ζ chain associated with the detergent-insoluble fraction is distributed between cytoskeleton as well as lipid-rich membrane microdomains, composed primarily of sphingolipids and cholesterol, and an enriched subset of proteins that float laterally as “rafts” within the plasma membrane⁴⁵. Lipid rafts are preformed functional modules that serve as platforms for signal transduction and membrane trafficking. Recent data indicate that lipid rafts are crucial for effecting TCR signal transduction^{18, 35}. TCR engagement leads to translocation and concentration of tyrosine phosphorylated TCR ζ chain and downstream signal transduction molecules within lipid rafts²⁶. Conversely, perturbation of the structural integrity of lipid rafts inhibits TCR-induced protein tyrosine phosphorylation and Ca^{2+} flux^{35, 65}.

Dissociation of the lipid-rafts by cholesterol depletion using methyl-β-cyclodextrin showed increased percentage of the residual membrane bound TCR ζ chain in the lipid-rafts in resting SLE T cell membranes (NAMBIAR et al., Arthritis Rheum., in press, Jan. 2002). Fluorescence microscopy indicated that the residual TCR ζ chain is more clustered on the cell membranes of SLE T cells compared to normal. Faster kinetics of TCR/CD3-mediated $[Ca^{2+}]_i$ response in SLE T cells also supports the clustering or cross-talk between signaling pathways in SLE. Upon TCR/CD3 activation ζ chain clusters became more prominent in SLE T cells and they superimpose with LAT, suggesting that they are co-localized to lipid rafts. Increased lipid-raft association and surface clustering of the residual TCR ζ chain may explain the supernormal TCR/CD3-mediated $[Ca^{2+}]_i$ response in SLE T cells. Based on these data we have proposed a model (Fig. 3) that suggests that in SLE T cells, although there is a deficiency, the residual TCR ζ chain is more associated with membrane lipid rafts resulting in more preformed TCR clustering.

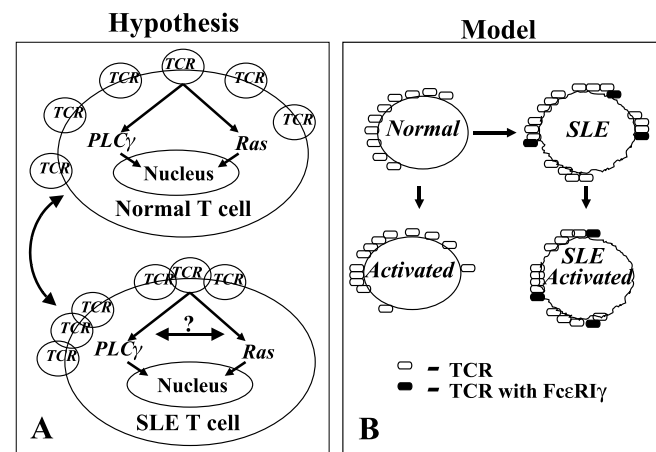


Fig. 3. Proposed hypothesis (A) and experimental model (B) for the molecular mechanisms involved in TCR/CD3-mediated supranormal $[Ca^{2+}]_i$ response in SLE. **A** – owing to the rapid kinetics of TCR/CD3-mediated signal transduction in SLE compared to normal T cells, we hypothesized that, faster kinetics must require clustering of signaling molecules or cross-talk between inter or intra-signaling pathways. **B** – experimental evidence suggests that the deficient TCR ζ chain is replaced by another family member FcεRIγ chain generating functionally diverse TCR. TCR substituted with a FcεRIγ chain may signal transduce more efficiently because the downstream signaling molecule Syk is 100-fold more potent than ZAP-70. In addition, increased membrane lipid raft association of the residual TCR ζ chain and TCR ζ chain clustering suggests that molecular defects in SLE T cells leads to preformed functional TCR clusters. Because TCR/CD3-mediated signaling requires supra-molecular assembly of TCR, preformed functional clusters promote TCR/CD3-mediated $[Ca^{2+}]_i$ response in SLE T cells

Increased membrane TCR clustering as well as replacement of the deficient TCR ζ chain by FcεRIγ chain could lead to supranormal $[Ca^{2+}]_i$ response under TCR ζ chain deficiency and decreased tolerance in SLE T cells. In support of this view, recently, it has been reported that mice with N-acetylglucosaminyltransferase deficiency show decreased glycosylation of T cell membrane proteins that prevent galectin binding and thereby disrupting the galectin-glycoprotein lattice, increased clustering of TCR⁷. Increased TCR clustering in these autoimmune mice had a very similar phenotype of human SLE, with lowered T cell activation thresholds and increased TCR signaling⁷. Similarly, it has also been suggested that genetic remodeling of protein glycosylation by mutation of α mannosidase II induces autoimmune disease⁴. As the association between the residual ζ chain with the lipid rafts, TCR clustering and T cell effector functions is further explored, it is likely that important new insights will emerge that will explain ζ chain abnormalities and autoreactivity of T cells.

Deficient Protein Kinase A-I Activity

Studies on adenylyl cyclase/cAMP/protein kinase A (AC/cAMP/PKA) system, a key metabolic pathway integral to cellular homeostasis, identified impaired cAMP-dependent protein phosphorylation in SLE T cells³². Abnormal cAMP-dependent signaling pathway is associated with deficient CD8 suppressor T cell function and altered cytoskeletal regulation of CD3, CD4, and CD8 receptor mobility within the plane of the plasma membrane^{20, 22}. Recently, it has been demonstrated that abnormal cAMP-dependent signaling pathway reflects a profound reduction in PKA-I isozyme²¹. Deficient PKA-I activity is the consequence of reduced type I regulatory subunit. Deficient T cell PKA-I activity reflects reduction of both holoenzymes $RI\beta_2C_2 > RI\alpha_2C_2$ ²⁹. Deficient PKA-I activity possibly contributes to altered T cell effector function by altering the protein phosphorylation that regulates cellular pathways that promote cell growth and differentiation. Similar to TCR ζ chain increased mutation/polymorphism of PKA-I regulatory subunit α has been reported in a SLE patient²⁸. The kinase activity of PKC⁴⁹ and Lck is also impaired in SLE T cells³³. The activity of other kinases such as protein kinase PKR that is involved in the phosphorylation of translation initiation factors is increased in SLE T cells¹⁴.

Interrelationship between the Ca^{2+} and AC/cAMP/PKA pathway raise the possibility that defective PKA activity in part contribute to the impaired Ca^{2+} homeostasis. TCR/CD3-mediated increase in IP3 and $[Ca^{2+}]_i$ is downregulated by inactivation of PLC γ 1 through PKA dependent phosphorylation⁴⁰. Deficient PKA-catalyzed phosphorylation may retain the activity of PLC γ 1 and contribute to the supranormal and sustained $[Ca^{2+}]_i$ response in SLE T cells.

Decreased Ras-MAP Kinase Signaling and DNA Hypomethylation

Environment factors including drugs and ultraviolet light induce SLE-like disorder. Because these agents induce hypomethylation of DNA and modify the expression of the affected genes a relationship between DNA hypomethylation and SLE has been sought. Up-regulation of lymphocyte function-associated antigen 1 (LFA-1/CD11a) generates autoreactive state by exaggerated help for the production of autoantibodies. The activity of the DNA methylation enzyme DNA (cytosine-5)-methyltransferase (Dnmt) appears to be regulated in part by the Ras-MAP kinase pathway and it has

been suggested that the reduced Dnmt-I activity in SLE T cells is a function of deficient Ras-MAP kinase signaling⁸. SLE T cells show about 50% reduction in Dnmt-I activity and mRNA content and activation by PMA resulted in reduced Ras-MAP kinase catalyzed protein phosphorylation. Currently, the mechanisms responsible for impaired Ras-MAP kinase activity in SLE T cell remain to be elucidated. The functional impact of impaired signaling kinases would be underphosphorylation of transcription factors that regulates their conformation and DNA binding ability resulting in the modulation of gene expression.

Abnormalities in Transcription Factor Expression

Early signaling abnormalities are followed by altered activation of transcription factors and abnormal gene transcription. It is notable that while certain genes are transcribed at low rates (the TCR ζ chain and IL-2 genes), others are transcribed at increased rates (the genes for the γ chain of the Fc receptor for IgE and the CD40L)⁵⁶. In addition to the TCR ζ chain transcription factor Elf-1 described above, defects have been identified both in the expression/activation of other transcription enhancers and repressors including NF- κ B p65-Rel A subunit and p-CREB.

Deficient p65-Rel a Subunit of NF- κ B in SLE T Cells

The transcription factor, NF- κ B plays a profound role in immune and proinflammatory responses and IL-2 production¹². The possibility that reduced IL-2 production by SLE T cells may be a product of altered NF- κ B activity has been analyzed by electrophoretic mobility shift assays⁶⁴. NF- κ B activity in the nuclear extracts is significantly decreased in SLE T cells. In the group of SLE patients with decreased NF- κ B activity, the transcriptionally active, heterodimeric p65/p50 complex was not formed in the cytosol. The deficiency of NF- κ B heterodimeric complex could be responsible for the down-regulation of IL-2 and may have extensive pathophysiological significance in the expression of the disease.

Increased p-CREB Binding to the -180 Site of the IL-2 Promoter

SLE T cells stimulated *in vitro* in response to antigens or mitogens proliferate significantly less than

T cells from normal donors⁴⁸. Activated SLE T cells also secrete low IL-2 *in vitro* that may reflect increased T cell anergy. Another mechanism for the low IL-2 production by SLE T cells is inhibition of IL-2 enhancer/promoter transcriptional activation. Cyclic AMP response element modulator (CREM) and inducible camp early repressor (ICER) are two transcriptional repressors that bind to camp response elements (CREs) and downregulate genes containing this binding site. Recently it has been demonstrated that phosphorylated CREM (p-CREM) binds to the -180 region of the IL-2 enhancer/promoter and contributes to T cell anergy⁴¹. This raised the question that p-CREM upregulation may contribute the downregulation of IL-2 secretion and contribute T cell anergy. Nuclear extracts from resting SLE T cells showed significantly increased binding of p-CREM/p-CREB to the -180 site of the IL-2 enhancer/promoter⁴⁶. Some patients revealed both p-CREM and p-CREB, although p-CREM was the main factor. We have found that activated normal T cells increase p-CREB binding by 10-fold in contrast to negligible p-CREM binding to the -180 site. By contrast, activated SLE T cells bound both p-CREM and p-CREB in a 2:1 ratio. Thus, preferential phosphorylation and predominant occupancy of the -180 site of the IL-2 enhancer/promoter by p-CREM during activation may hamper optimum transcriptional activation by p-CREB binding resulting in very low IL-2 production in SLE T cells. In contrast to the heteromeric complex formation between p-CREB to two nuclear co-activators, CREB-binding protein (CBP) and p300, that initiate transcription in normal T cells, SLE T cells mainly form p-CREM/CBP/p300 complexes that may suppress the IL-2 synthesis. Current studies using real-time PCR aimed at establish the prevalence, show that majority of the SLE patients with low IL-2 level reveal increased CREM mRNA (TENBROCK et al., unpublished results).

Abnormal Nuclear Translocation of RII β Subunit

The RII β subunit of PKA-II is not classified currently as a nuclear factor, although its translocation to nucleus and binding to CREB supports the role of a transcription factor. Upon activation of the PKA-II holoenzyme, RII α/β_2C_2 by reversible cAMP binding to the RII subunits release RII β that reversibly translocates to nucleus. Although it has been suggested that nuclear RII β binds CREB, at present its function in the nucleus still remains uncertain. RII β subunit can be

constitutively in the nucleus suggesting an ongoing activation of RII β_2C_2 during homeostasis. The mechanism of deficient PKA-II activity in SLE T cells differs from PKA-I, and involve increased nuclear translocation of the RII β to the nuclear fraction, diminishing the capacity to form RII β_2C_2 holoenzyme³⁴.

Abnormal CD40L Expression

Some of the molecular abnormalities identified in SLE can readily be linked to known defects. For instance, heightened calcium responses can explain the increased expression of CD40L and other molecules whose expression depends on calcium-controlled transcription factors. Because both T and B cell express CD40 it is plausible that increased expression of CD40L on the surface of T cells is responsible for the increased interaction between T cells and B cells^{5, 25, 56, 57}.

Restoration of Signaling Abnormalities by Gene Therapy

Extensive efforts over the past decade identified several inter-related components of the signaling pathway is intrinsically impaired. It is likely that additional molecular abnormalities will be discovered in the near future. Restoring the function of these molecules may correct the signaling abnormalities and open new avenues for the treatment of SLE. We have developed an efficient method using green fluorescence protein under the control of cytomegalovirus promoter to transiently transfect the T cells. The method essentially utilizes overnight resting of peripheral blood mononuclear cells under low concentration of phytohemagglutinin (PHA) and IL-2 that is sufficient to promote cell proliferation and then isolation of T cells by negative depletion using magnetic beads and electroporation of the gene of interest. Electroporated cells were maintained in low concentration of PHA/IL-2 and analyzed at different time points. By using this approach we demonstrated that SLE T cells transfected with TCR ζ chain significantly increased the level of IL-2 secretion compared to mock transfected cells. Immunoblotting data indicated that these transiently transfected cells partially restored the deficient expression the NF- κ B p65 subunit (NAMBIAR et al., unpublished data). This data was further supported by the fact that, direct expression of NF- κ B p65 subunit by transient transfection

significantly restored the IL-2 reporter gene activity in SLE T cells (HERNDON et al., submitted for publication). Similarly, transient transfection of full-length cDNA RI β cDNA construct under the control of a cytomegalovirus promoter to SLE T cells also demonstrated a significant increase in the cAMP-activatable PKA specific activity. Upon stimulation, these transiently transfected SLE T cells augmented the production of IL-2²³. These findings suggest that it is possible to transiently transfect and restore the function of defective genes and repair the signaling abnormalities. These observations also suggest that restoring the function of single gene promotes the expression of other defective genes to a certain extent by cross-talk or unknown mechanisms that help to bypass the overall signaling defect and enhance IL-2 secretion in SLE T cells. Complete restoration of the IL-2 production could be achieved by supplementing all the defective genes suggesting that the wave of the future is combination gene therapy or a central gene that can restore multiple signaling abnormalities.

Summary and Future Perspectives

SLE is a challenging disease with varied manifestations resulting from widespread immune complex deposition. Our understanding of the role of genetics and environmental agents in the pathogenesis of SLE has improved over the past ten years. In addition, the past ten years have seen tremendous improvements in identifying molecular defects in immune cells to unravel the mechanism of autoimmunity. Emphasis has been placed on identifying the genetic basis of these molecular defects and the restoration of their functions. Abnormalities of transcription factors contribute to immune response by linking signaling pathways and gene expression. Identification of several signaling defects suggests the successive components of the signaling cascade may be intrinsically defective in SLE T cells. Important concern regarding the cellular and molecular pathogenesis is to seek all the molecular defects at the cellular level and establish the genetic basis for this complex autoimmune disease in a timely fashion. Despite the multitude of abnormalities in SLE, reconstitution of the molecular defects promise pharmacological intervention that may serve to blunt the diverse clinical manifestations of the disease and improve quality of life and survival.

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