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Targeting lymphocyte signaling pathways as a therapeutic approach to systemic lupus erythematosus

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Abstract

Purpose of review—Over the past year several key pathways in systemic lupus erythematosus (SLE) lymphocyte signaling have been identified. Pathways that can be exploited for therapy are discussed in this review.

Recent findings—Inhibition of SLE T cell activation by blocking spleen tyrosine kinase (Syk) and SLE T cell migration by blocking CD44 or CXCR4 lead to amelioration of lupus in lupus-prone mice. Similar results can be achieved by boosting CD8+ Treg numbers. Small molecules that block the kinases CaMKIV (calcium and calmodulin dependent kinase IV) and Bruton Tyrosine kinase (Btk) and the phosphatase calcineurin were shown to be effective in treating murine lupus. Finally, gene methylation status determines the expression of several key genes in SLE and strategies to correct it have shown promising results in preclinical studies.

Summary—Molecules that enhance T cell receptor (TCR) signaling or increase lymphocyte migration can be inhibited successfully with significant improvement of disease intensity in lupus-prone mice using small molecules. Manipulation of promoter methylation and histone acetylation represents a novel way to alter gene transcription in SLE.

Keywords

epigenetics; lymphocytes; systemic lupus erythematosus (SLE); treatment

Introduction

During the past year, several key pathways in the activation of systemic lupus erythematosus (SLE) lymphocytes have been identified. Herein, we provide evidence that targeting surface or intracellular molecules in SLE lymphocytes can lead to amelioration of disease. We also address the observed epigenetic changes in SLE that clearly affect the expression of signaling molecules and approaches to restore epigenetic aberrancies found in SLE.

Surface molecules

T cells from SLE patients display a distinct phenotype that is characterized by the over-expression and clustering of several adhesion and co-stimulatory molecules into cholesterol-rich membrane domains called lipid rafts. In addition, the T cell receptor complex is rewired

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Conflicts of interest
There are no conflicts of interest.

with the CD3ζ chain being substituted by the FcRγ chain. FcRγ chain recruits spleen tyrosine kinase (Syk), a very efficient signal transducer instead of the canonical ZAP70 kinase [1]. Due to these changes, SLE T cells once activated display an early and robust protein tyrosyl phosphorylation [2] and calcium influx [3] that enables them to invade tissues and provide excessive help to B cells. This pathway can be specifically targeted using the Syk inhibitor R788 (fostamatinib), which showed promising results for the treatment of rheumatoid arthritis [4]. Following in-vitro experiments, which had shown that Syk inhibition normalize SLE T cell calcium responses [5], MRL/*lpr* lupus prone mice were fed with R788 at two doses [6•]. R788 treated MRL/*lpr* mice (with either dose) did not develop secondary lymphoid organ hyperplasia and were protected from skin and kidney inflammation. R788 also had an effect on reducing disease activity even when administered in animals with already established disease. Syk is expressed primarily in monocytes and therefore its effectiveness in murine lupus may also be due to inhibition of monocyte lineage cells as well. Indeed, the authors show a reduction of CD11c+ cells infiltrating the skin of the treated mice. Somewhat surprisingly since B cells express Syk, the authors did not observe a reduction of anti-dsDNA antibodies in the treated animals. Overall, this study suggests that Syk is a promising therapeutic target in SLE. Further trials will be needed to address the patterns of expression of Syk in SLE and its role in autoantibody production.

SLE T cells have an increased capacity to invade target tissues such as the kidneys and skin. This is may be due to the increased expression of the surface glycoprotein CD44 that binds hyaluronic acid and increased levels of phosphorylated ezrin, radixin, and moesin (ERM), as it was shown in a small cohort of SLE patients [7]. Moreover CD44+pERM+ T cells were observed in the kidney biopsies of patients with lupus nephritis. Inhibiting the Rho kinase, which phosphorylates ERM, resulted in ‘halting’ SLE T cells. Intriguingly inhibition of Rho kinase in a mouse model of lupus resulted in amelioration of disease by inhibiting the production of interleukin-17 and interleukin-21 [8]. Following this observation, Crispin *et al.* [9] evaluated the expression patterns of CD44 in 76 SLE patients and equal number of controls. They showed that two variants of CD44, v3, and v6, are not only expressed at higher levels on the surface of SLE T cells as compared with control T cells, but also that their expression positively correlates with the presence of nephritis, dsDNA, and overall SLE disease activity. This important finding suggests that CD44 may be an important therapeutic target in SLE, as its inhibition may avert homing of pathogenic T cells in target tissues.

On the contrary, though CD44 together with Ly49 and CD122 is expressed on the surface of CD8+ Treg cells that help eliminate T follicular helper cells (TFH) [10••]. TFH cells play a crucial role in the propagation of the autoimmune humoral responses [11]. CD8+ Treg cells were found to be defective in the murine lupus model B6-Yaa possibly contributing to the development of autoimmunity through excessive B cell activation. In this setting, targeting CD44 may prove counter-productive as TFH activity and autoantibody production/immune complex formation will undoubtedly increase. Further studies especially in patients rather than animal models of SLE should be done to elucidate the precise interplay of CD8+ Treg, TFH and B cells and the role CD44 plays not only in T cell homing but also in immune regulation.

Given their role in preventing autoimmunity, factors that support the survival/expansion of these CD8+ Treg cells may be of particular importance in the treatment of lupus. Previous work showed that expansion of CD8+ Treg cells during tolerization of New Zealand black × New Zealand white F1 (BWF1) mice was associated with decreased expression of programmed death (PD)-1 [12]. The role of PD-1 in regulating CD8+ Treg was further elucidated by inhibiting PD-1 in BWF1 mice using a neutralizing antibody [13•]. The animals were protected from the development of lupus and maintained a functional

suppressive CD8+ T cell population. Interestingly the same anti-PD-1 antibody when administered in the context of tolerizing strategies prevented tolerization, suggesting that PD-1 inhibition may prove difficult to incorporate in SLE treatment regimens.

As mentioned above, migration of lymphocytes in tissues is important for the pathogenesis of SLE. Following observations in murine lupus [14], Wang *et al.* [15] showed that SLE T and B cells over-express the C-X-C chemokine receptor type 4 (CXCR4). This was more pronounced in naïve CD4+ and CD19+ cells. CXCR4 binds to circulating chemotactic factor Chemokine (C-X-C motif) ligand 12 (CXCL12) and leads to increased capacity of T cells to migrate to tissues. In addition, CXCR4 plays a role in B:T cell cross-talk. CXCL12 was detected in the kidneys of patients with lupus nephritis suggesting that in-situ production of this chemokine coupled with upregulation of the chemokine receptor on lymphocytes leads to sequestration of lymphocytes in target organs. The importance of CXCR4-CXCL12 in lupus was demonstrated in a trial [14] of a peptide inhibitor of CXCR4 that led to significant improvement of lupus nephritis in lupus prone animals. These studies pave the way for targeting the CXCR4-CXCL12 pathway in patients with SLE.

Intracellular targets

Calcium influx following T cell receptor (TCR) engagement leads to the activation of calcineurin, which dephosphorylates Nuclear Factor of Activated T cells (NFAT). The dephosphorylated NF-AT is responsible for the enhanced transcription of multiple early response genes such as CD154, a key co-stimulatory molecule in T:B cell cross-talk and production of T cell dependent autoantibodies in SLE. Similar to SLE patients, MRL/*lpr* mice display before the onset of clinical disease a hyperactive T cell phenotype, with robust calcium influx upon T cell activation [16•]. Over time, as the mice get older and sicker, MRL/*lpr* T cells accumulate NFATc1 as they become activated, invade tissues, and provide help to B cells. Besides cyclosporine and tacrolimus that inhibit calcineurin, dipyridamole, a widely used antiplatelet agent, was recently identified as an inhibitor of calcineurin-NFAT interaction. Indeed dipyridamole inhibited the activation of SLE T cells *in vitro*, limited the production of T cell cytokines, and blocked T cell dependent production of immunoglobulin by B cells without affecting the B cells directly. Importantly, MRL/*lpr* mice treated with dipirydamole had delayed onset of clinical disease and lower level of interleukin-6 in their peripheral blood.

High calcium flux in SLE T cells is accompanied by activation of the calcium and calmodulin dependent kinase IV (CaMKIV), which contributes to decreased expression of interleukin-2 [17] by SLE T cells. Treatment of MRL/*lpr* mice with KN-93, a small molecule inhibitor of CaMKIV, resulted in amelioration of both nephritis and skin disease [18]. KN-93 treatment affected primarily TNF-α and interferon-γ (IFN-γ), suggesting a possible central role of CaMKIV in murine lupus.

The aberrant signal transduction in SLE is PP2A, a serinethreonine phosphatase that negatively regulates the interleukin-2 gene transcription in SLE by inactivating (dephosphorylating) c-AMP response element binding protein (CREB) [19]. Interestingly Protein phosphatase 2A (PP2A) was found to activate by dephosphorylation specificity protein 1 (Sp1) [20]. Sp1 binds to the promoter of c-AMP response element modulator (CREM) and increases its expression. This observation suggests that PP2A is central in deregulation of the transcriptional balance between enhancer p-CREB and repressor CREM tipping the balance toward CREM and leading to interleukin-2 decreased expression.

SLE and murine T cells invading the kidneys, produce IFN-γ, which is important in mediating kidney damage [4]. IFN-γ binds to its receptors and activates the Jak/Stat pathway. Therefore, MRL/*lpr* mice were treated with the Jak2 inhibitor AG490 for 12

weeks. The effect of this compound was a moderate decrease in renal damage that was accompanied by decreased infiltration of the kidneys by T cells and monocytes. These data suggest that targeting the local effects of immune mediators in the kidney may prove to be an effective stand-alone or adjunct treatment in SLE.

SLE is characterized by the over-production of (auto) antibodies by abnormal B cells. Immunoglobulin production is greatly enhanced by the engagement of CD40 on B cells by CD154, which as stated above is over-expressed on SLE T cells. The ubiquitin modifying enzyme A20 prevents the over-excitation of B cells when CD40 is engaged. Mice that lack A20 develop autoimmunity characterized by glomerulonephritis [21,22] and autoantibody production. These data suggest that targeting A20 can compliment or even substitute CD154 targeting in SLE.

B cell receptor mediated B cell activation is also amplified by Bruton Tyrosine kinase (Btk). Therefore Btk is an attractive candidate for decreasing B cell over-activity in SLE. A selective nonreversible inhibitor of Btk, PCI 32765 is currently in development for the treatment of B cell lymphoma [23••]. MRL/*lpr* mice were treated with PCI 32765 for 12 weeks starting at 8 weeks, before disease onset. There was a modest decrease in antibody production and kidney inflammation as measured by proteinuria and level of glomerulonephritis. Btk inhibition therefore provides an alternative way to suppress autoantibody production in SLE.

Epigenetic changes in T cells

It has been increasingly recognized over the last few years that epigenetic factors that alter DNA and histones may decisively influence the immune system. Methylation of gene promoters and acetylation of histones determine the transcriptional activity of genes. It has been known from several studies that the promoters of CD70 [24,25] and CD11a [26] are hypomethylated in SLE. These two molecules play a significant role in T cell, and T cellinduced B cell hyperactivity in SLE. In two recent studies, the Regulatory factor X-box 1 (RFX1) was found to be a negative epigenetic regulator of these genes. RFX1 binds to DNA methyltransferase 1 (DNMT-1), histone deacytalase (HDAC) [27], and histone methyltransferase SUV39H1 [28], and enables epigenetic modifications of CD70 and CD11a genes. As RFX-1 expression is decreased in SLE [27], the promoters of both CD70 and CD11a become more accessible to transcription factors and gene transcription is enhanced.

Methylation is also important for the regulation of the promoter of PP2Ac, an important culprit of SLE T cell dysfunction. PP2ac promoter was found to be hypomethylated in SLE T cells [19]. This promoter hypomethylation allows the binding of p-CREB to the promoter and results in increased gene transcription.

The methylation status of the promoter not only correlated with the level of gene transcription. As was the case with CD70 and CD11a, PP2Ac hypomethylation is probably related to DNMT-1 as there was a correlation between PP2Ac mRNA and DNMT-1 mRNA.

These studies provide preliminary evidence that DNMT-1 is a significant intermediate in the aberrant gene transcription of lupus T cells and therefore a potential treatment target. Besides RFX-1, micro RNAs (miR) regulate DNMT-1. Specifically miR-126 was found to correlate with DNMT-1 levels in SLE T cells and directly target the 3′-UTR (untranslated region) of the DNMT-1 mRNA. When overexpressed, miR-126 boosted the expression of both CD70 and CD11a [29•]. Using a different cohort of patients as well as lupus prone mice, Pan *et al.* [30] identified miR-21 and miR-148a as repressors of DNMT-1 leading to decreased transcription of CD70 and CD11a. miR-21 indirectly targets DNMT-1 through

modulation of the Ras-Mitogen Activated Protein Kinase (MAPK) pathway. miR-148a is a direct inhibitor of DNMT-1. Given the limited number of patients examined in these studies, the exact role of microRNAs in regulating DNMT-1 is still unclear, but do provide a novel target for therapeutic intervention in SLE.

Besides DNMT-1, the growth arrest and DNA damage-induced 45a (GADD45α), a global de-methylator was found to be increased in SLE T cells as well as in normal CD4+ cells exposed to ultraviolet radiation [30]. The investigators showed through overexpression and silencing experiments that GADD45α de-methylates the promoters of CD70 and CD11a, leading to an increase in the protein expression and enhanced T:B cell cross-talk. These sets of experiments provide an initial link between functionally relevant epigenetic changes in lupus T cells and ultraviolet light, a known instigator of lupus flares.

Genes are also regulated posttranscriptionally by proteins that bind to their 3′-UTR. CD3ζ expression is decreased in SLE T cells partly because of the expression of an abnormally unstable splice isoform. Alternative splicing factor/splicing factor 2 (ASF/SF2) was recognized as a factor that preferentially binds to the wild type CD3ζ mRNA and boosts its production while limiting the expression of a defective alternative splice isoform [31]. Boosting the expression of ASF/SF2 may therefore lead to normalization of the TCR complex in SLE T cells and correct their abnormal response to external stimuli.

Conclusion

We presented evidence that altering key signaling pathways at the membrane or cytoplasmic levels using a variety of approaches can ‘correct’ the phenotype of SLE lymphocytes and in some instances improve disease activity in lupus-prone animal models. Moreover, recent studies have argued that altering epigenetic changes in SLE may be a novel approach to treatment. Future work should focus on translating the findings in in-vitro experiments and lupusprone mice into strategies for treatment of SLE.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 499).

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Key points

- Spleen tyrosine kinase (Syk) is aberrantly expressed on SLE T cells and its inhibition ameliorates murine lupus.
- SLE T cells display increased levels of cell surface molecules that enable them to migrate to tissues under the direction of chemokines.
- CD8+ Treg may represent a significant counter-regulator of autoimmunity.
- SLE T and B cell kinases and phosphatases, such as CaMKIV, calcineurin, PP2A, and Btk alter the T cell receptor (TCR)/B cell receptor (BCR) signaling intensity.
- Epigenetic changes play a significant role in gene transcription in SLE T cells.