T cells as therapeutic targets in systemic lupus erythematosus

José C. Crispín, Vasileios C. Kyttaris, Cox Terhorst and George C. Tsokos

Abstract | T cells contribute to the initiation and perpetuation of autoimmunity in systemic lupus erythematosus (SLE), and seem to be directly involved in the development of related organ pathology. Defects associated with CD8+ and T-regulatory (T<sub>reg</sub>) cell function manifest in parallel with the expanded erythematosus (SLE), and seem to be directly involved in the development of related organ pathology. Amplification and perpetuation of end-organ damage. Approaches that limit the cognate interaction between T cells and B cells, prevent inappropriate tissue homing and restore T<sub>reg</sub> cell function and the normal cytokine milieu have been entertained. Biochemical characterization of SLE T cells has revealed distinct early and late signaling aberrations, and has enabled the identification of novel molecular targets that can be corrected with small molecules, and biomarkers that may foretell disease activity and predict organ damage.


Introduction

T cells in patients with systemic lupus erythematosus (SLE) display altered attributes and have an important role in disease progression and pathology. SLE T cells show inappropriate tissue homing and promote inflammation by secreting cytokines and activating dendritic cells and B cells. Although the precise circumstances that facilitate the ignition of SLE-related pathology remain mostly unknown, evidence indicates that, once tolerance is broken, T cells and B cells participate in the amplification and perpetuation of the autoimmune and inflammatory response. Both the cellular and humoral regulatory mechanisms fail to stop this process, while inflammation and tissue injury further contribute to the amplification and perpetuation of end-organ damage.

Corticosteroids and immunosuppressive drugs are commonly used to treat patients with SLE. As indiscriminate suppressors of the immune-mediated inflammatory response, they mitigate pathology and clinical disease activity—albeit at the cost of considerable adverse effects. In this Review, we present the advances made during the last decade that have shed light on the biochemical and molecular aberrations that lead to the characteristic cytokine production pattern and altered behavior of SLE T cells. These findings help us to understand the nature of the SLE T cell, point to therapeutic targets that deserve further development, and identify additional, much-needed biomarkers in patients with SLE.

Cell signaling aberrations

T cells are equipped with a variety of receptors that enable them to acquire a comprehensive representation of the situation in which an antigen is being presented. According to signals delivered during its presentation, an antigen can elicit the differentiation of T lymphocytes into proinflammatory cells, T-regulatory (T<sub>reg</sub>) cells, or quiescent, anergic T cells. The correct interpretation of external stimuli, such as cytokines and surface-bound molecules, is, therefore, central to the development of an appropriate response. This section discusses alterations to various aspects of T-cell signaling in SLE.

Altered CD3–TCR signaling

T cells from patients with SLE display faster and increased early signaling events upon engagement of the CD3–T cell receptor (TCR) complex (Figure 1 and Box 1). These events include an increase in intracellular calcium levels and phosphorylation of tyrosine residues in cytoplasmic proteins, which contribute to the abnormal function of SLE T cells. For example, increased intracellular calcium levels may lead to increased expression of CD40 ligand (CD40L) and increased levels of the transcriptional repressor CREM (cAMP response element modulator).

TCR rewiring

The altered calcium response has been shown to be a consequence of the reorganization of the proximal signaling machinery of SLE T cells. Levels of CD3ζ, the principal signaling molecule in the CD3–TCR complex, are lower in T cells from patients with SLE than in those from healthy individuals. Decreased CD3ζ levels are concomitant with the appearance of an analogous molecule, FcRγ (the common γ chain of the Fcε receptor), which is normally scarce in T cells. Importantly, FcRγ partners with Syk (spleen tyrosine kinase) instead of its normal signaling molecule [Au:OK?] ZAP-70 (ζ-chain-associated protein...
Aggregation of lipid rafts

Lipid rafts are cholesterol-rich areas of the membrane where signaling molecules accumulate at high density. Lipid raft clustering is evident in freshly isolated T cells from patients with SLE that have not been manipulated in vitro. \( ^{12, 13} \) Signaling in T cells from patients with SLE is further facilitated by the clustering of lipid rafts, as cross-linking of the gangliosides present in lipid rafts prior to the engagement of the TCR further augments the signaling response. \( ^{12} \) The importance of this phenomenon is supported by the fact that a lipid-raft-clustering agent (cholera toxin) accelerated disease progression in a mouse model of lupus, whereas methyl-\( \beta \)-cyclodextrin, a substance that disrupts raft clustering, delayed the appearance of disease. \( ^{14} \) Accordingly, manipulation of lipid raft aggregation on SLE T cells might represent a process susceptible to therapeutic targeting. \( ^{15} \)

Increased T-cell death

Spontaneous apoptosis is increased in T cells from SLE patients. \( ^{16} \) On the other hand, upon exposure to oxidizing agents, SLE T cells undergo necrosis rather than apoptosis. \( ^{17} \) These abnormalities have been attributed to mitochondrial hyperpolarization and increased levels of reactive oxygen intermediates in these T cells—changes that alter the expression of redox-sensitive cytokines (such as interleukin [IL] -10 and tumor necrosis factor). \( ^{18} \) Increased T-cell necrosis and apoptosis represents a source of nuclear material, which amplifies the inflammatory response in patients with SLE.

Elevated expression of CD44

Expression levels of CD44, a cell-surface molecule involved in cell adhesion, migration, and signaling, are abnormally high in T cells from patients with SLE. \( ^{12, 19} \) Moreover, CD44 is found within aggregated lipid rafts along with its signaling partners, the ERM (ezrin, radixin, and moesin) proteins (Figure 2). T cells with increased levels of phosphorylated ERM proteins are found in kidney infiltrates of patients with SLE, \( ^{12} \) suggesting that high expression levels of (and increased signaling through) CD44 facilitates the migration of SLE T cells into inflamed tissues. Among the variant CD44 isoforms, CD44v3 and CD44v6 are increased in patients with SLE and correlate with disease activity and the presence of nephritis and anti-DNA antibodies. \( ^{20} \) Accordingly, CD44v3 and CD44v6 might serve as markers of organ involvement and disease activity.

Altered gene transcription

A number of large-scale genome-wide association studies have identified several genetic loci that harbor genes assumed to be associated with the development of SLE. \( ^{21- 24} \) However, none of the genes involved in the biochemical abnormalities described above (CD44, CD247 and FCER1G) have appeared in these studies, a fact indicative of the complexity of the disease. Although polymorphisms in certain genes can predispose an individual to SLE, the presence of these polymorphisms does not always result in the phenotypic variations
Box 1 | Altered signaling molecules and transcription factor abnormalities in SLE T cells

<table>
<thead>
<tr>
<th>Signaling molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased PKC activity</td>
</tr>
<tr>
<td>Decreased PKA levels and activity</td>
</tr>
<tr>
<td>Increased calcium response</td>
</tr>
<tr>
<td>Decreased CD45 phosphatase activity</td>
</tr>
<tr>
<td>Increased phosphorylation of tyrosine residues</td>
</tr>
<tr>
<td>Decreased expression of CD3ζ</td>
</tr>
<tr>
<td>Decreased activity of Lck</td>
</tr>
<tr>
<td>Increased expression of FcRγ</td>
</tr>
<tr>
<td>Decreased MAPK activity</td>
</tr>
<tr>
<td>Increased activity of PI3K</td>
</tr>
<tr>
<td>Increased lipid raft clustering</td>
</tr>
<tr>
<td>Increased expression and activity of Syk</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transcription factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased NFκB nuclear activity</td>
</tr>
<tr>
<td>Increased binding of phosphorylated CREM to the IL-2 promoter</td>
</tr>
<tr>
<td>Decreased binding of phosphorylated CREB to the IL-2 promoter</td>
</tr>
<tr>
<td>Decreased nuclear translocation and DNA binding of AP1</td>
</tr>
<tr>
<td>Decreased levels of functional ELF1</td>
</tr>
<tr>
<td>Increased levels and nuclear translocation of NFATc2</td>
</tr>
</tbody>
</table>

DNA methylation

DNA methylation, which represents an important epigenetic mechanism involved in the suppression of gene expression, is decreased in T cells from SLE patients and results in overexpression of several proteins, including PP2A, CD70, and CD40L. Altered signaling, particularly via decreased mitogen-activated protein kinase (MAPK) activity, has been proposed to have a role, as this pathway regulates DNA methyltransferase activity. Elevated levels of the proinflammatory cytokine IL-6 have also been shown to contribute to decreased DNA methylation in B cells from patients with SLE.

T cell subsets

According to the presence or absence of the coreceptors CD4 and CD8, TCRαβ+ T cells can be divided into CD4+, CD8+, and double negative (DN; CD4+CD8+) populations (Figure 3). CD4 and CD8 expression is determined in the thymus, whereas at least some of the DN T cells derive from a subset of activated CD8+ T cells.

CD8+ and DN cells

The role of CD8+ T cells in SLE is not well determined. Patients with active SLE have increased numbers of
activated CD8+ T cells, and CD8+ T cells are present in the kidneys of patients with lupus nephritis. On the other hand, deficient CD8+ cytotoxic capacity has been reported in SLE. In patients with the disease, the TCRαβ+ DN T cells are expanded, and may originate from CD8+ T cells that have lost surface CD8 molecules following stimulation with anti-CD3–TCR autoantibodies and autoantigens. DN T cells infiltrate target organs (such as the kidneys), produce proinflammatory cytokines (including IL-18 and IL-17), and promote B-cell antibody production.

**Tfh cells**

CD4+ T cells undergo functional differentiation after antigen priming, which grants them a distinct phenotype in terms of transcriptional regulation and cytokine production (Figure 3). Type 17 helper T (Th17) cells are CD4+ T cells that express the transcription factors RORγt and RORα and produce the cytokines IL-17A, IL-17F, IL-21, and IL-22. They induce intense inflammatory responses by secreting IL-17, which is a powerful neutrophil attractant. Patients with SLE and lupus-prone mice have increased numbers of IL-17-producing T cells and elevated serum levels of IL-17. Furthermore, IL-17-producing T cells have been observed among inflammatory infiltrates in kidneys affected by SLE. IL-17 can also stimulate B-cell antibody production, and has been shown to participate in the formation of germinal centers in lupus-prone mice.

**Treg cells**

Treg cells represent a constitutively suppressive CD4+ cell subset, characterized by the expression of the transcription factor FOXP3. Treg cells are necessary for the maintenance of immunological tolerance, and their absence causes a spontaneous and fatal autoimmune disorder. Treg cell numbers are low in patients with SLE, and their suppressive function is compromised. Some reports have highlighted the fact that SLE effector T cells may be particularly resistant to Treg-induced suppression. In the context of SLE, Treg cell deficiencies are probably related to the decreased production of IL-2, a cytokine that is essential for their development and survival, and to elevated levels of proinflammatory cytokines, particularly IL-6, which can inhibit Treg cell function. Treg cell defects can contribute to SLE pathology by failing to control the response of other T cells and autoantibody production. Patients with SLE who achieve remission have high levels of nonconventional Treg cell populations. Accordingly, therapeutic approaches to correcting IL-2 production or suppressing IL-6 levels seem worthy of consideration.

**T-cell-targeted therapeutic approaches**

**Cell-based treatment**

Autologous hemopoietic stem cell transplantation (HSCT) has been shown to induce long-term remission in patients with SLE refractory to standard therapies. In post-transplant patients, CD4+ CD25highFOXP3+ T cells, as well as an unusual subset of CD8+ FOXP3+ Treg cells, were shown to return to levels observed in normal individuals, accompanied by inhibited T-cell responses to histone peptides. This clinical information, along with the report that infusion of natural Treg cells in lupus-prone mice can suppress autoimmunity, prompts further consideration of the potential of cell-based treatment in SLE.

**Cognate cellular interactions**

**CTLA4–Ig (abatacept)**

For optimal T-cell stimulation, the engagement of both the TCR and CD28 molecules with their receptors on
the antigen-presenting cell is needed. Inhibition of the CD28–CD80/86 interaction would result in suboptimal activation of the T cell, despite adequate TCR engagement with the MHC–antigen complex. The activated T cell can end its own stimulation by expressing CTLA4 (cytotoxic T-lymphocyte antigen 4; also known as CD152) on its surface. CTLA4 binds to CD80/86 with significantly higher affinity than CD28, thus out-competing the CD28 and preventing further T-cell activation. Abatacept (a CTLA4–Ig fusion molecule) was constructed with the aim of preventing the CD28–CD80/86 interaction. This agent was shown to be effective in improving disease activity in lupus-prone mice, especially when given in combination with cyclophosphamide. Abatacept combined with background mycophenolate mofetil treatment is currently being administered to patients with lupus nephritis in a phase II/III clinical trial. Another phase II trial of abatacept in patients already receiving cyclophosphamide and/or azathioprine for lupus nephritis is also under way.

**CD40L**

CD4+ and CD8+ T cells, as well as B cells, overexpress CD40L in patients with SLE. Preclinical studies in lupus-prone mice with nephritis showed that treatment with an anti-CD40L antibody resulted in decreased disease severity and increased survival when compared to placebo-treated mice. Two different antibodies against human CD40L (BG9588 and IDEC-1) have been used in patients with SLE. Treatment with BG9588, but not IDEC-1, resulted in improvement of serologic markers of disease activity (such as decreased anti-dsDNA titers, increased C3 concentration and decreased hema
tus). Unexpectedly, anti-CD40L treatment resulted in thrombocytopenic events, possibly through binding of the antibody to CD40L on the surface of activated platelets. As a result, clinical trials of these antibodies are currently in abeyance. The aberrantly increased expression of CD40L by B cells might be abrogated by other pharmacologic agents that target the calcium influx and/or the phosphatase calcineurin; these agents should not result in platelet aggregation and thrombotic events.

**ICOS–B7RP1**

Activated T cells express ICOS, which binds to B7-related peptide 1 (B7RP1; also known as inducible T-cell co-stimulator ligand [ICOSLG]). T cells use this pathway to induce class switching and IgG production by B cells. When an antibody against B7RP1 was injected into mice with lupus nephritis and inflammatory arthritis, it resulted in significant disease amelioration. One proposed mechanism for this observation is the inhibition of the development of T<sub>FH</sub> cells, which help B cells in the germinal centers. A fully humanized anti-B7RP1 antibody (AMG557) is being evaluated in phase I trials with SLE.

**LFA1–ICAM1**

LFA1 (lymphocyte function-associated antigen 1) expressed by activated T cells binds to ICAM-1 (intercellular adhesion molecule 1; also known as CD54) expressed on endothelial cells and stabilizes the immune synapse. The monoclonal humanized IgG1 antibody efalizumab, which targets the a-subunit of LFA1 (CD11a), has been used successfully in cases of severe cutaneous lupus erythematosus. However, the association between efalizumab treatment and the development of progressive multifocal leukoencephalopathy in a number of patients led to its withdrawal from the market.

**Cytokines**

**IL-6**

Monocytes secrete IL-6, which, together with transforming growth factor β, promotes the differentiation of T<sub>H</sub>17 cells. IL-6 levels are elevated in lupus-prone mice and in the serum and urine of patients with SLE, and can be detected in kidney biopsies of patients with lupus nephritis. Inhibition of IL-6 or IL-6 receptor (IL-6R) using specific antibodies resulted in clinical improvements in lupus-prone mice, including decreased anti-dsDNA...
antibody titers and proteinuria, and improved survival. The fully humanized monoclonal anti-IL-6R antibody tocilizumab has shown promise in a phase I trial of SLE [Au: I think the reference cited here (Mihara, M. et al. Clin Exp Immunol 112, 397–402 [1998]) was not the correct one. I have added Illei G. G. et al. Arthritis Rheum. 62, 542–552 (2010) to the reference list and cited it here, as I think this is the study you mean. OK?]. The treated patients had decreased levels of acute-phase reactants and small decreases in serum immunoglobulin and anti-dsDNA levels. However, as two patients developed severe neutropenia, further studies are needed to address the efficacy and toxicity of this biologic agent in patients with SLE.

**IL-17, IL-21 and IL-23**

As discussed above, evidence stipulates the involvement of the cytokines IL-17, IL-21 and IL-23 in the development of SLE pathology both in mice and humans. Germline-targeted deletion of IL-21 or IL-23 receptor resulted in significant mitigation of autoimmunity and glomerulonephritis, strongly supporting the consideration of these molecules as potential therapeutic targets in SLE. An anti-CD3 antibody administered intranasally to lupus-prone SNF1 mice resulted in induction of CD4+CD25+LAP+ Treg cells and suppression of IL-17+CD4+CXCR5+ Tfh cells. As noted above, decreased Treg cell function and increased numbers of Tfh cells have been implicated in the pathogenesis of SLE. More information is needed to demonstrate the efficacy of anti-CD3 antibodies in other strains of lupus-prone mice, and whether the route of administration is important.

**Signaling**

**Syk**

Given the central role of Syk in the transduction of signals from various immune receptors and its involvement in SLE, an oral Syk inhibitor, fostamatinib (R788), was developed. Fostamatinib is a prodrug that is metabolized to the active compound R406. This drug was shown to be effective at reducing disease activity in a phase II trial of patients with rheumatoid arthritis, and promising preclinical data have emerged from studies in lupus-prone mice and from *in vitro* studies of SLE T cells. R788 treatment of lupus-prone NZB/W F1 and MRL/lpr mice resulted in delayed disease onset and prolonged survival. Additionally, complementary work showed that R406 is able to block calcium influx in SLE T cells, thus correcting one of the major consequences of the altered TCR composition in SLE T cells.

**mTOR**

Sirolimus (also known as rapamycin) is a lipophilic macrolide antibiotic that alters mitochondrial transmembrane potential and calcium fluxing, and is used primarily to control transplant rejection. Sirolimus binds FKBP1B and the regulatory kinase mTOR (mammalian target of rapamycin). This drug corrected some of the biochemical alterations of SLE T cells, including the decreased expression of CD3ζ. Sirolimus was shown to prevent anti-dsDNA antibody production and glomerulonephritis and to prolong survival in lupus-prone mice. Sirolimus also showed promising clinical results in nine patients with SLE.

**CAMK4–CREM**

Capitalizing on improvements of cellular transfection techniques, several key molecules in the abnormal signaling cascade in SLE T cells have emerged as potential therapeutic targets. CAMK4 has been shown to be a key molecule in the downregulation of IL-2 in activated SLE T cells. When T cells were transfected with a plasmid encoding a dominant-negative form of CAMK4, the enzymatic activity of CAMK4 was blocked and the production of IL-2 was boosted via inhibition of CREM binding to the IL2 promoter.

T cells transfected with an antisense plasmid that suppressed production of CREM showed significantly increased production of IL-2 and c-Fos, thus establishing the importance of the CAMK4–CREM pathway in the downregulation of IL-2 in SLE T cells. Gene therapy approaches that aim to suppress CREM and CaMK4 expression might be deserving of consideration as therapeutic approaches.

**Adhesion**

SLE T cells invade tissues via upregulation of surface adhesion molecules and rearrangement of their cytoskeleton. The enhanced adherence and migratory ability shown by SLE T cells is mediated by upregulated CD44. *In vitro* experiments have shown that silencing the expression of CD44 with specific small interfering RNA limits SLE T cells’ ability to adhere and migrate. The same outcome could be accomplished by blocking the phosphorylation of ERM (the intracellular signaling partners of CD44) by specific inhibition of Rho-kinase (ROCK), the kinase that activates ERM (Figure 2). Similarly, targeting the cytoskeletal rearrangement mechanism by blocking actin polymerization with cytochalasin D substantially impaired the ability of SLE T cells to adhere and migrate.

**Conclusions**

T cells from patients with SLE display a fascinating phenotype that defies classification along the lines defined by the study of normal lymphocytes. Both at the cellular and cytokine level, SLE T cells present an antithetic signature. Although T cells can help B cells to produce autoantibodies at increased levels, they fail to generate cytotoxic and Treg function. At the cytokine level, although they fail to produce IL-2, they release increased amounts of IL-6 and IL-17. Decreased levels of IL-2 may account for the suppressed cytotoxic responses and Treg cell function and decreased elimination of autoreactive T cells through activation-induced cell death in patients with SLE. IL-17 is produced by several T-cell lineages, including CD4+ and CD3+CD4+CD8– T cells, which are expanded in SLE patients and are present in inflamed kidneys. CD44 seems to be expressed at increased levels on SLE T cells, and might account for their inappropriate tissue homing.
Advances in our understanding of the molecular workings of SLE T cells have begun to explain the SLE T-cell phenotype. Increased CD3–TCR-mediated calcium responses might, via increased NFAT levels, explain the increased expression of CD40L. Decreased production of IL-2 seems to be the result of increased CAMK4 expression, which causes increased binding of CREM to the IL-2 promoter, and increased PP2A expression, which leads to dephosphorylation of pCREB. The molecular cause of increased IL-17 production remains unknown. T-cell-targeted therapeutic approaches include blockade of T cell–B cell interactions, restoration of IL-2 and suppression of IL-17 production, and limiting the tissue-homing potential of T cells.

Identification of the unique biochemical abnormalities that seem to account for the aberrant T-cell function, such as increased Syk, CAMK4, and PP2A expression, calls for the development of small-molecule kinase/phosphatase inhibitors. Small-molecule inhibitors of Syk have been shown to suppress SLE pathology in animal models. Similarly, specific inhibitors of CaMK4 and PP2A may prove to have similar effects and deserve clinical testing. By virtue of restoring early and late T-cell signaling processes, these small-molecule drugs might correct effector T-cell function and prove of clinical value in patients with SLE.


Review criteria
The references included in this Review were obtained from the authors’ collection of articles about the pathogenesis and treatment of systemic lupus erythematosus pathogenesis.


