T-cell-directed therapy in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with significant morbidity and mortality that commonly afflicts young women. It ranges from a mild disease with a predominance of joint and skin manifestations to a severe life-threatening disease that can have serious CNS or renal involvement. While there are several immune effector mechanisms that contribute to the disease, it is clear that T lymphocytes have a central role in the immune pathogenesis of SLE. There are five broad approaches to T-cell-directed therapy in SLE that will be reviewed, including the use of monoclonal antibodies or decoy receptors directed against cytokines or cell surface costimulatory molecules; the use of synthetic peptides to selectively modulate autoantigen reactive T cells; cell-based therapies; restoration of abnormal T-cell receptor signaling events; and the correction of epigenetic abnormalities found in SLE. The current and future feasibility of each of these approaches to treatment are discussed.

Decoy receptors or anticytokine mAbs
Decoy receptors & mAbs directed against cytokines or cell surface costimulatory molecules
The use of decoy receptors to bind circulating tumor necrosis factor (TNF-α) in serum and the use of mAbs that bind circulating TNF-α are well established, highly effective therapies in the treatment of rheumatoid arthritis and other inflammatory diseases [4]. Similar approaches directed against cytokines important in T-cell function in SLE may have benefit as therapy.

Preliminary, uncontrolled clinical trials suggest that a decoy receptor that binds TNF-α, etanercept (Enbrel™), may be used safely in SLE; furthermore, small, uncontrolled clinical series and case reports using anti-TNF therapy in SLE suggest that it may have efficacy in reducing disease activity [5]. The proinflammatory cytokine interleukin (IL)-6 has been found to be elevated in the serum of patients with SLE and genetic polymorphisms of the
IL-6 promoter are associated with susceptibility to SLE in family and population studies [6]. Targeting IL-6 in SLE is a T-cell-directed therapy with potential merit. Currently, there is a registered ongoing clinical trial examining the use of anti-IL-6 in SLE [101]. Controlled trials are needed to definitively address the role anti-TNF-α or anti-IL6 therapy may have in the management of SLE. However, based upon experience in rheumatoid arthritis, inflammatory bowel disease, psoriasis and the spondyloarthropathies, targeting proinflammatory cytokines with mAbs or decoy receptors is an approach that has substantial merit and warrants further research [4]. In addition, it is important to acknowledge that this is an area
where there is already substantial technical experience in both generating and using such reagents. Thus, based upon these facts, rapid progress could potentially be achieved if the correct cytokine(s) were identified for targeting in SLE.

Costimulatory receptor blockade

Abnormalities in costimulation are a general mechanism that can, in theory, break immune tolerance and lead to autoimmunity [3]. There is substantial evidence that some costimulatory pathways are abnormal in SLE. Crow and colleagues, Datta and colleagues, and subsequently other investigators have identified impaired regulation of expression of CD40L in T cells from patients with SLE [7,8]. Other costimulatory receptors could theoretically play key roles in the pathogenesis of SLE through excess costimulation. Interestingly, candidate gene studies examining the programmed cell death receptor 1 gene (PD1) in SLE have reported association between an intronic single nucleotide polymorphism and susceptibility to disease among some populations [9,10]. PD-1–PDL1, PD-2–PDL2, ICOS–ICOS ligand and other costimulatory molecules and their ligands may be overexpressed in SLE, and could be targeted to block excess T-cell activation in SLE [11]. Safety concerns, however, remain paramount, particularly for cell surface molecules with wide tissue distribution, in view of recent experience with the IDEC-131, anti-CD40Ligand (CD40L; also known as CD154) mAb [12].

Evidence for the concept of blocking costimulatory receptors on T cells has now been published in studies that used a monoclonal antibody (IDEC-131) to successfully block CD154 on T cells and modulate disease activity in SLE [12,13]. Unfortunately, the use of IDEC-131 has been associated with serious adverse events, including death in some of the clinical trials of SLE, and, therefore, clinical trials with abatacept in SLE are ongoing [102].

Use of synthetic peptides to modulate autoantigen-reactive T-cell immune response

It is has been demonstrated that T cells from the peripheral blood of patients with SLE can be identified that react against a variety of self-antigens, including many autoantigens that have been implicated in the pathogenesis of SLE; these are summarized in Box 2 [3]. T cells reactive with a variety of lupus-associated nuclear autoantigens, including U1-70kD, small nuclear ribonucleoprotein, DNA-histones, the small nuclear ribonucleoproteins Sm-B, Sm-D and U1-A, and heterogeneous ribonucleoprotein (hnRNP) A2 have all been isolated from the peripheral blood of SLE patients and characterized. Selective targeting of these autoantigen-reactive T cells is another potential novel approach to therapy in SLE [3].

One of the best characterized T-cell responses against an autoantigen in SLE is that against uridine-rich, small nuclear ribonucleoproteins that comprise the spliceosome [3,14–16]. These small nuclear ribonucleoproteins are evolutionarily highly conserved, ubiquitous self antigens that are components of the spliceosome complex, which normally functions to excise intervening introns and generate mature messenger RNA transcripts in eukaryotic cells [2,3]. T cells isolated from patients that are reactive with the U1-70kD antigen have been reported to be CD4+ T cells, express the α/β TCR, and produce interferon (IFN)-γ, IL-2, IL-4 and IL-10 [3,14,15]. They can provide help ex vivo for anti-U1-70kD and anti-hnRNP antibody production and their presence is closely linked to the presence of autoantibody-producing cells in the serum of the same specificity. T-cell epitope mapping studies of human T cell reactive with the U1-70kD small nuclear ribonucleoprotein autoantigen revealed that T cells were directed solely against a functional region of the protein, known as the RNA binding domain [3,14,15]. Furthermore, a murine model has recently been developed by immunizing nonautoimmune mice with the...
All of the T-cell epitopes identified in this model reside within the RNA binding domain of U1-70kD and these are sufficient, when injected with U1-RNA into nonautoimmune-prone human leukocyte antigen (HLA)-DR4 transgenic mice, to induce SLE-like autoimmune disease [16]. Furthermore, in the MRL/lpr animal model, which spontaneously develops an SLE-like illness, Muller and colleagues have demonstrated that T-cell reactivity is directed primarily against the RNA binding domain of U1-70kD, similar to human disease and the inducible animal model [3,14–16]. Thus, in all cases studied of human disease and murine models, anti-U1-ribonucleoprotein, autoreactive T cells are directed against the RNA binding domain of U1-70kD. Taking advantage of these observations, Monneaux and Muller have recently determined that by injection of a peptide analog encompassing residues 131–151 (within the RNA binding domain) of U1-70kD, and modified by phosphorylation of the serine residue at position 140 of the molecule, they could prolong disease survival in the MRL/lpr murine model of SLE [17]. Furthermore, they report that the phosphorylated peptide inhibited proliferation, but not cytokine secretion, by human peripheral blood mononuclear cells (PBMC) from patients, suggesting that the phosphorylated peptide might act as an activator of regulatory T cells or as a partial T cell receptor (TCR) agonist in modulating human disease [18]. Clinical trials of this modified peptide are anticipated (Muller S, Pers. Comm.).

**16/6 idiotype synthetic peptide**

A second peptide-based approach for which there is preliminary clinical trial work is the use of a synthetic peptide GYYWSWIRQPPG-KGEEWIG, named EdratideTM (NCT00203151) [19,20]. Edratide is based upon the identification of an idiotype (Id) contained within the complementarity determining region (CDR)-1 of Ig heavy chain sequence of a human anti-DNA autoantibody, first named 16/6 [19,20]. It has been demonstrated in mice that immunization with human CD1 16/6 sequence concomitant with induction of TGF-β, and suppression of 16/6-induced T-cell proliferation by downregulating the adhesion molecules

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**Box 1. Approaches to T-cell-directed therapy.**

- Monoclonal antibodies or decoy receptor
  - Target cytokine (e.g., interleukin-6)
  - Target costimulatory molecules (e.g., CD28)
- Synthetic peptide
  - Autoreactive T cells U1-70kD RNA binding domain modified peptide
  - Synthetic peptide modulation of autoantigen reactive T cells (e.g., immunoglobulin CDR1-based peptide and EdratideTM)
- Cell-based therapies
  - Autologous or allogeneic cell transplantation (e.g., CD34+ cells with T-cell depletion)
  - Cell vaccination
  - Regulatory cell replacement (e.g., infusion of CD4+CD25+FoxP3+ T cells)
- Correction of T-cell signaling defects
  - Gene therapy to correct CD3-zeta deficiency
  - Caspase inhibitor
  - Protein kinase C θ replacement
- Epigenetic abnormalities corrected
  - Histone deacetylase inhibitors to correct DNA hypomethylation in T cells

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**Box 2. T cells reactive with the following autoantigens have been isolated from peripheral blood of patients with systemic lupus erythematosus autoantigen.**

- U1-70kD small nuclear ribonucleoprotein
- Double-stranded DNA
- Histones
- Sm-B small nuclear ribonucleoprotein
- Sm-D small nuclear ribonucleoprotein
- U1-A small nuclear ribonucleoprotein
- Heterogeneous ribonucleoprotein A2
CD11a/CD18 (LFA-1) and CD44. Furthermore, immunization with Edratide results in downregulation of TNF-α, IFN-γ and IL-10 and amelioration of disease in murine models of SLE. A Phase II, randomized, double-blind, placebo-controlled, parallel assignment safety/efficacy study examining the tolerability, safety and effectiveness of Edratide in the treatment of lupus is now in progress. Taken together, the U1-70kD peptide and 16/6 peptide (Edratide) are two promising examples of how synthetic peptides may be utilized to modulate T-cell immune responses in SLE.

Restoration of normal T-cell signaling pathways

Another well-characterized abnormality of T cells in SLE is defective TCR signaling. A series of signal transduction events following TCR complex engagement have been shown to be abnormal in SLE and are summarized in Box 3 [21–29]. Tsokos and colleagues have characterized a series of defects in intracellular signal transduction pathways in T cells from patients with SLE that were based upon the early observation that there is decreased IL-2 production from T cells in SLE. The work of Takeuchi and colleagues has emphasized the importance of proximal signaling defects and their effects on subsequent downstream signaling defects [25,26]. They and others have demonstrated exaggerated intracellular calcium responses, abnormal intracellular phosphorylation and decreased TCR CD3-zeta chain expression [3,21–29]. Therapeutic approaches in SLE potentially include correcting these T-cell signaling abnormalities [21–26]. Tsokos and colleagues have shown in vitro that CD3-zeta chain expression, with enhanced signaling responses and IL-2 production could be normalized by gene transfer and with caspase-3 inhibitors. Other proposed approaches to normalize T-cell IL-2 production include modulation of the cyclic AMP (cAMP)-protein kinase pathway and replenishing protein kinase C (PKC) (Box 1).

Cell-based therapies including hematopoietic cell transplantation

Cell-based therapies used to modify or restore T-cell immune function are currently being tested in clinical trials in patients with SLE (e.g., [103–106]). These involve a variety of approaches, including the use of either autologous or allogeneic bone marrow transplantation, autologous hematopoietic stem cell transplantation with or without T-cell-depletion conditioning regimens prior to cell infusions and autologous cell ‘vaccination’ [103–106]. In addition, the currently registered clinical trials of transplantation in SLE that are ongoing in the USA utilize a variety of conditioning regimens, some of which specifically deplete T cells prior to cell transfer. For example, one of these depletes T cells prior to cell transfer. For example, one of these depletes T cells using CAMPATH anti-T-cell mAb prior to cell transfer [104], while others use antithymocyte globulin as part of the conditioning regimen prior to and/or immediately following cell transfer [30,31]. While these approaches to cell-based therapy appear to have merit, especially for patients with severe life-threatening disease, mortality remains high and the treatment protocols themselves differ substantially, making comparison between studies difficult. Controlled trials with significant numbers of patients are yet to be published.

A second cell-based therapeutic approach that has been published for immune tolerance induction utilized autologous T cells to ‘vaccinate’ SLE patients [32]. An uncontrolled, open-label study of six patients from China reported clinical improvement in SLE, as measured by SLE disease activity index (SLEDAI) at 32–40 months following the subcutaneous administration of irradiated, autologous autoreactive T cells of unknown specificity given four-times over an 8-week period [32]. In this study, the SLE patients who received vaccination with irradiated autologous, autoreactive T-cell clones were found to have no adverse reactions and all six remained in clinical remission at the time of the follow-up. The manipulation of autoreactive T cells and manipulation of immune networks in SLE have been discussed further in the section above.

Finally, there has been intense interest in the role of regulatory T cells in autoimmunity in recent years [33–39]. In animal models, adoptive transfer of syngenic and allogeneic regulatory T cells has been found to modify autoimmunity. Several groups have reported that CD4+CD25+ T cells are reduced in active SLE [33–37]. To our knowledge the first published full-length report

Box 3. T-cell receptor signaling abnormalities identified in systemic lupus erythematosus that might be targeted for therapy abnormalities.

- Exaggerated intracellular calcium responses
- Abnormal intracellular phosphorylation
- Decreased T-cell receptor/CD3-zeta chain expression
- FcRγ chain upregulation
- Syk recruitment to T-cell receptor complex
in SLE was by Crispin and colleagues, who reported that CD4+CD25+ peripheral blood T cells were reduced in number compared with healthy controls and SLE patients without active SLE [33]. Subsequently, Liu and colleagues published that CD4+CD25+ T cells were decreased in SLE, but the levels of these cells did not correlate with disease activity in their study [34]. Arguably, the most comprehensive and elegant study of CD4+CD25+ T-regulatory cells in SLE to date has been that of Miyara and colleagues, who reported that the depletion of CD4+CD25+ T cells in peripheral blood did correlate with flares of disease as measured using the SLEDAI, and that this was due to global depletion of CD4+CD25+ T cells in that they did not find that this depletion in peripheral blood could be attributed to redistribution of these cells to the kidney or peripheral lymph nodes [35].

However, a major barrier to the application of cell-based therapy using autologous regulatory cells has been the inability to expand these cells ex vivo. Promisingly, Bluestone and colleagues have recently reported that they have been able to expand CD4+CD25+ T-regulatory cells ex vivo [36,37]. They also report success with in vivo expansion of T-regulatory cells and modulation of Type I diabetes mellitus in nonobese diabetic mice [38,39]. Thus, this remains a promising area of investigation and one that has the potential to yield substantial new approaches to immune regulation, either through successful expansion and infusion of autologous regulatory cells or through enhancing our current underlying concepts of immune tolerance leading to identification of novel approaches to therapy.

Correction of epigenetic abnormalities
Richardson and colleagues have proposed that epigenetic abnormalities in SLE may have significant importance in pathophysiology [40,41]. They have shown that overexpression of the accessory molecule LFA-1 (also known as CD11a/CD18) can be found in spontaneous and drug-induced SLE, and that, in experimental models, overexpression of LFA-1 via DNA hypomethylation induces autoreactivity and a lupus-like syndrome [40]. They have suggested that epigenetic modification of DNA, such as by hypomethylation by exogenous chemicals (e.g., procainamide) or other currently unidentified mechanisms in idiopathic SLE, may be of fundamental importance in the pathogenesis of disease [41]. Interestingly, they have linked abnormal T-cell signaling and excess costimulation of T cells with DNA hypomethylation, showing that CD4+ T cells from patients with active SLE, characterized by defective extracellular signal-regulated kinase signaling and increased CD70 expression, can have similar abnormalities induced by a panel of drugs that result in DNA hypomethylation. Thus, if the hypothesis that epigenetic modification of DNA is important in the pathogenesis of SLE is correct, then appropriate modification or normalization of DNA methylation may hold promise as a unique approach to T-cell-directed therapy [40,41].

Conclusion
There are a number of approaches that have substantial promise as new T-cell-directed therapies in SLE; these include the use of mAbs and decoy receptors directed against cytokines, and mAbs directed against costimulatory molecules. Studies in animal models and in vitro studies suggest that peptide-based therapies, correction of TCR signaling abnormalities or correction of epigenetic abnormalities may also have promise as T-cell targeted therapies. Finally, cell-based therapies are already in use, but there remain substantial barriers to their application; however the clinical use of regulatory T cells, holds significant promise. In the near-term, anticytokines have proven efficacy in a number of rheumatic diseases, and mAbs against costimulatory molecules have also have demonstrated clinical efficacy; these appear to be the approaches that have the greatest immediate promise in the treatment of patients with SLE.

Future perspective
Over the next 5–10 years, we can anticipate that there will be continued progress in the use of anticytokine mAb and decoy receptors against cytokines and blocking of costimulatory molecules as emerging therapies in SLE. It remains to be seen whether a single target can be identified that will substantially impact the disease, as has been the case for anti-TNF-α and anti-CTL4 therapies in rheumatoid arthritis. The redundancy of the immune system may require targeting multiple cytokines or costimulatory molecules in SLE, which will be technically more difficult and may be substantially more expensive. Finally, studies on T-regulatory cells are rapidly advancing our understanding of immune tolerance, and this area holds substantial promise for developing new approaches to manipulate immune tolerance and control autoimmunity. We may anticipate that this area will be fruitful in the more distant future.
### Executive summary

**T cells play a central role in immune function & in the pathogenesis of systemic lupus erythematosus**

- Excess cytokine production is found in systemic lupus erythematosus (SLE).
- Excess costimulation is demonstrated in SLE.
- Autoantigen-reactive T cells have been identified in SLE.
- Defective T-cell signaling abnormalities have been defined in SLE.
- Epigenetic abnormalities that influence T-cell function have been identified in SLE.

**Several approaches hold promise as T-cell-directed therapy in systemic lupus erythematosus**

- The following approaches hold promise as T-cell-directed therapies in SLE:
  - Monoclonal antibodies and decoy receptors directed against cytokines or costimulatory molecules.
  - Peptide-based therapies that modify autoantigen-specific immune responses.
  - Correction of T-cell receptor signaling abnormalities.
  - Cell-based therapies.
  - Correction of epigenetic abnormalities.

### Decoy receptors & monoclonal antibodies

- Decoy receptors or monoclonal antibodies are therapies directed against cytokines or cell surface costimulatory molecules; antitumor necrosis factor (TNF), anti-interleukin (IL)-6, anti-CD40 and cytotoxic T-lymphocyte-4 immunoglobulin are all examples of such therapies.
- Synthetic peptides have been used to to modulate autoantigen reactive T-cell immune responses; U1-70kD modified peptide and 16/6 idiotype-derived peptide from human immunoglobulin CDRI are examples of peptide-based therapies.
- Restoration of T-cell signaling pathways is another approach of novel T-cell therapy; correct TCR-ζ defect by gene transfer, transfect cAMP response element modulator (CREM) and protein kinase C replacement are examples of this approach.
- Cell-based therapies are another approach; autologous or allogeneic cell transplantation, T-cell vaccine and T-regulatory cell manipulation are examples of this approach.
- Correction of epigenetic abnormalities, such as through the use of histone deacetylase inhibitors, may have merit if epigenetic abnormalities do play a role in the pathogenesis of SLE.

### Conclusion

- Several T-cell-directed therapeutic approaches have substantial promise.
- The most rapid progress is anticipated in the use of anticytokine monoclonal antibodies and decoy receptors, based upon the advanced state of these in other diseases.
- It is currently unknown whether targeting a single cytokine or costimulatory molecule will be effective in SLE, but IL-6, tumor necrosis factor-α and B7/CD28 have been identified as targets.
- T regulatory cells have more distant promise as either therapy or by providing fundamental insight into immune tolerance and autoimmunity.

### Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

• Large clinical trial demonstrates the efficacy of using an adhesion molecule in the treatment of a systemic autoimmune disease, rheumatoid arthritis.


** Demonstrates that the antiribonucleoprotein U-1-70kD autoimmune response is directly pathogenic in a new animal model of systemic autoimmunity.


** Demonstrates that a phosphorylated peptide can modulate CD4 cells in vitro in systemic lupus erythematosus (SLE).


** Demonstrates that Id therapy with a synthetic peptide can ameliorate disease in a murine model of lupus and that it does so via influences on T-regulatory cells.


** Demonstrates the influence of caspase-3 on T-cell receptor expression.


** Demonstrates that CD4+ and CD25+ T regulatory cells are depleted during active SLE.


** Demonstrates the manipulation of T-regulatory cells can influence remission of autoimmune diabetes in an animal model of disease.


T-cell-directed therapy in systemic lupus erythematosus – REVIEW

Websites
101. SLE Clinical trial number: NCT 00119678
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