SUPPLEMENT

Systemic lupus erythematosus: new molecular targets

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T cells from patients with systemic lupus erythematosus exhibit a notable array of defects that probably contribute to the origin and development of the disease. Such abnormalities include an abnormal response to stimulation, aberrant expression of molecules that play key roles in intracellular signalling pathways, altered transcription factor activation and binding, and skewed gene expression. The combination of these alterations leads the cell to the expression of a particular phenotype that intense research has gradually uncovered over the last years. The aim of this article is to review the findings that have allowed us to better understand the behaviour of the lupus T cell and highlight the molecules that represent potential therapeutic targets.

T cells play a central role in the development and regulation of immune responses. They stimulate and suppress through mechanisms that involve direct and indirect actions on several cells, including antigen-presenting cells and B lymphocytes. Such faculty allows them to regulate the specificity, and also the magnitude and duration of most immune responses.¹ T cells from patients with systemic lupus erythematosus (SLE), a generalised autoimmune disease, exhibit a notable array of defects that probably contribute to the origin and the development of the disease.² Several abnormalities that have been consistently reported in T cells obtained from patients with SLE have allowed us to craft a conceptual model that describes the odd behaviour of the lupus T cell and partly explains its defects.³ Evidence indicates that it has several phenotypic and functional characteristics that distinguish it from normal T cells. However, the presence of different aberrations is heterogeneous and different patients are expected to have different combinations of such defects. Thus, it is probable that each patient with SLE has a distinctive arrangement of alterations that could make her prone to specific disease manifestations and simultaneously, candidate to different therapies. In this sense, the concepts that have arisen during the last decade are of paramount importance and will permit us to understand the disease pathogenesis and to be able to design rational targeted therapies adjusted to the particularities of each individual patient. The aim of this article is to review such findings and their conceptual meaning, and highlight the molecules that represent potential therapeutic targets.

ABNORMAL T CELL ACTIVATION MECHANISMS

T cells from patients with SLE behave in an abnormal manner when stimulated through their specific receptor (TCR). Such phenotype includes a lower excitation threshold as well as an abnormally high intracellular calcium response. The increment in the intracellular calcium levels is more rapid and reaches a higher level than in normal T cells. This was one of the first T cell abnormalities reported to be specific to lupus T lymphocytes.⁴ The aberrant kinetics of the calcium response parallels an increase, in magnitude and speed, in the tyrosine phosphorylation of proteins involved in the proximal part of the TCR/CD3 transduction pathway.^{5–6} However, for several years after these alterations were described reasonable mechanistic explanations were lacking. A significant discovery followed: T cells from a high proportion of patients with SLE (approximately 80%) lack a component of the TCR-associated

signalling complex, the CD3 ζ chain;⁷ the defect is found both at the protein and mRNA levels.^{3–8} Further work has demonstrated that in lupus T cells a surrogate molecule takes the place of the missing CD3 ζ chain. A molecule normally absent in T cells, the Fc receptor (FcR) γ chain (originally identified as a component of the Fc ϵ R1), is found associated with the TCR/CD3 complex instead of the CD3 ζ chain.⁹ The impostor is fully functional and its presence leads to the establishment of an alternative signalling transduction pathway. It undergoes phosphorylation following CD3 ζ cross-linking and associates with Syk, a kinase normally absent in normal T cells but abundantly expressed in lupus T cells.¹⁰ The resultant FcR γ –Syk duet is known to signal approximately 100 times more effectively than the conventional couple ζ – ζ associated protein (ZAP-70).¹¹ Thus, this *rewiring* of the lupus TCR, where the FcR γ –Syk complex replaces the ζ –ZAP-70 complex, explains, at least in part, the aforementioned hypersensitivity to CD3-mediated stimulation.^{3–10} The former notion was proven with the demonstration that the artificial expression of FcR γ in normal T cells mimics some of the changes described in lupus T cells, namely the augmented Ca²⁺ response to TCR stimulation and, interestingly, the decrease in the CD3 ζ chain levels.¹² Furthermore, treatment of SLE-derived T cells with piceatannol, at a concentration in which it exclusively inhibits Syk (20 μ M), led to the correction of the augmented calcium response and to a significant decrease in the presence of several abnormally phosphorylated proteins.¹⁰

The search for the mechanism responsible for the down-regulation of CD3 ζ in lupus T cells has proven to be a complex quest in which several underlying alterations have been found (box 1).^{13–18}

Initially, it became evident that decreased translation was involved. However, neither mutations nor polymorphisms have been found in the ζ chain promoter at the level of genomic DNA that could account for the reduced translation levels. Nevertheless, the ability of SLE T cells to drive the expression of a reporter gene carrying the wild-type promoter is clearly hampered. An alteration in the post-translational mechanisms that lead to the production of functional Elf-1 (E-74-like factor), has been found to be responsible for such a defect. Patients with SLE exhibit decreased production of the 98-KDa DNA binding form of this transcription factor.¹⁴

Further work has revealed that other abnormalities, particularly a conspicuous increase in the splice variation of the ζ chain mRNA, contributes to its decreased expression. Several splicing abnormalities have been found including splice insertion and splice deletion variants;¹⁹ the latter result from deletions of one

Box 1 Mechanisms of TCR ζ chain deficiency in SLE T cells

- Unstable TCR ζ chain mRNA – short 3'UTR
- Production of alternatively spliced (non-functional) TCR ζ chain mRNA
- Impaired translation of TCR ζ chain mRNA
- Impaired TCR ζ chain gene transcription
 - Decreased binding of the enhancer Elf-1
 - Increased binding of the repressor CREM
- Increased caspase-3-mediated proteolysis

or more exons. Reverse transcriptase (RT) PCR analysis has also revealed the existence of a novel ζ chain transcript with an alternatively spliced 3'-UTR region that is generated by the deletion of nucleotides from 672 to 1233 of ζ chain mRNA. This novel transcript is scarce in normal T cells but represents the dominant form of mRNA in SLE T cells. The ζ chain mRNA with alternatively spliced 3'-UTR region is less stable, and its presence leads to reduced expression of CD3 ζ .^{8, 13}

The mechanisms that decrease CD3 ζ chain expression in SLE T cells are not limited, however, to decreased transcription and dysfunctional splicing. An additional problem appears to play a role: the half-life of the CD3 ζ chain (measured as protein levels) is reduced.¹⁶ Several processes could account for such a phenomenon, but caspase-3-mediated proteolysis seems to be primarily involved. SLE-derived T cells have increased expression and activity of caspase-3, and CD3 ζ bears several caspase-cleaving sites in its cytoplasmic domain.^{20, 21} Treatment of T cells from patients with lupus with DEVD (a caspase-3 inhibitor) increases the cellular levels of the ζ chain without altering the expression of other closely related molecules (such as CD3 ζ). Interestingly, the same treatment does not cause any change in ζ chain levels in normal T cells (or in T cells from patients with Sjögren's syndrome), which suggests that caspase-3-mediated proteolysis does not regulate ζ chain levels in normal T cells.¹⁶

The reduction of the ζ chain levels, along with the reciprocal increase in the expression of FcR γ , is directly responsible for some of the phenotypic abnormalities characteristic of lupus T cells.²² Accordingly, replenishment of the ζ chain leads to the downregulation of the FcR γ chain and subsequently to the correction of the TCR/CD3-induced increased calcium response and the abnormal phosphorylation of cellular substrates.

Furthermore, it increases the production of IL2 (see below).²³ The former can be also achieved by the inhibition of caspase-3 activity.¹⁶

PRE-CLUSTERED LIPID RAFTS

The molecules that mediate signal transduction are distributed in a non-random fashion throughout the cell membrane; they are enriched within sections with high levels of cholesterol and gangliosides. These zones have been called lipid rafts and their function is to facilitate and coordinate close interactions between critical molecules in order to facilitate the amplification of downstream signalling events. In normal T cells, TCR ligation induces a rapid clustering of lipid rafts that leads to the concentration of signalling proteins at the immunological synapse.^{24, 25}

Membrane morphology and composition are altered in lupus T cells. They possess a larger quantity of lipid rafts, both in resting cells and after stimulation. Moreover, lipid rafts are already clustered in a large fraction of the lupus T cells despite the absence of an obvious stimulus.^{26–28} The aforementioned observation is congruent with the behaviour of SLE-derived T cells upon TCR-mediated stimulation. CD3 and LAT, among other molecules involved in signal transduction, are known to localise and to signal through lipid rafts.^{29, 30} Thus, the pre-clustered rafts could allow faster responses of a higher magnitude to occur. Further, the CD3 ζ chain is known to be associated with lipid rafts, and in fact in SLE T cells, even though the ζ chain is quantitatively diminished, the remaining fraction is predominantly localised within the membrane fractions that correspond to lipid rafts.²⁷ In addition, the molecular composition of the lipid rafts from T cells derived from patients with SLE shows several abnormalities. Apart from CD3 ζ and LAT (which are expected elements), FcR γ , active Syk kinase and PLC γ 1 are present.²⁶ These alterations have direct functional consequences and are probably directly linked to the heightened calcium response observed in SLE T cells.

Interestingly, some of the molecular abnormalities described in the preceding section, namely the decrease in the CD3 ζ chain and the increased caspase-3 activity, have been shown to be related to the pre-clustered lipid raft phenotype present in SLE T cells. Thus, the replenishment of the ζ chain has been shown to diminish the pre-clustering of lipid rafts in T cells from patients with SLE; blocking caspase-3 activity with specific inhibitors has a similar effect.¹⁶

INCREASED MIGRATION CAPACITY

The abnormal signalling pathways used by SLE T cells as well as the pre-clustered lipid rafts that allow faster and greater signal

Figure 1 The -180 site in the IL2 promoter is a key regulatory site. Binding sites for several transcription factors have been identified in the IL2 promoter. However, an imbalance of pCREB and CREM in the occupation of the -180 site plays a highly relevant role in the IL2 production defect characteristic of SLE T cells.

Figure 2 Abnormally increased expression of the phosphatase PP2A alters IL2 production in SLE T cells. The increased activity of PP2A limits transcription by two different mechanisms. It directly diminishes CREB phosphorylation and binding to the IL2 promoter, and it decreases AP-1 formation by altering *c-fos* production.

amplification grant an increased inflammatory capacity to the lupus T cell. Accordingly, the altered lipid raft composition outlined above is associated with an increase in the speed of actin polymerisation that follows CD3-mediated stimulation.¹⁰ Further, SLE T cells display an increased ability to adhere and migrate in response to chemotactic factors than T cells obtained from healthy individuals or from patients with rheumatoid arthritis.³¹ CD44 is a surface molecule that has been shown to participate in T cell adhesion and migration. It signals through a group of proteins (ezrin, radixin and moesin; ERM) that become phosphorylated on threonine residues and convey a signal that leads to formation of the uropod.³²

T cells from patients with SLE exhibit increased expression of CD44 and a parallel increase in the phosphorylation of ERM proteins (pERM). Furthermore, CD44, pERM and F-actin are distributed in a polar fashion in SLE T cells, forming caps that are normally only observed following cell stimulation. Such polar caps are indeed pre-clustered lipid rafts, and their presence depends on the phosphorylation of ERM proteins and the integrity of the cytoskeleton. Hence, treatment of SLE T cells with Y27632, a specific inhibitor of Rho kinase (the enzyme that phosphorylates ERM), disrupts the formation of polar caps and hampers the increased adhesion capacity characteristic of SLE T cells. The addition of cytochalasin D (an actin polymerisation inhibitor) or CD44 knockdown (by siRNA) has comparable effects.³¹ The importance of the former findings was highlighted by the demonstration that in kidney biopsies from patients with lupus glomerulonephritis, infiltrating T cells expressed CD44 and pERM. In contrast, biopsies from allografts undergoing rejection displayed CD44⁺ T cells, but lacked pERM expression.³¹

DECREASED IL2 PRODUCTION

IL2 has always been a puzzling cytokine. For years after its initial discovery it was regarded as a T cell-derived autocrine growth factor. It is considered an essential component of the T cell activation process. Its transcription begins soon after the T cell receives a productive stimulus and the T cell relies heavily on its presence in order to proliferate and develop effector functions. Nevertheless, evidence has shown that it plays a key role in the immune regulation process, such that its absence

leads to autoimmunity and not to immune deficiency as predicted earlier.³³ Interestingly, a phenotypic hallmark of the lupus T cell is a failure to produce normal amounts of IL2 upon activation.³⁴ Such deficiency can directly contribute to several of the disease characteristics such as decreased T cell responses, defective activation-induced cell death, and altered regulatory T cell homeostasis and function.^{35–37}

IL2 production is primarily controlled at the transcriptional level.³⁸ Sites for several transcriptional factors (NF- κ B, AP-1, NF-AT, CREB/CREM) have been described in the IL2 promoter, and optimal IL2 levels are only achieved when all the sites are occupied.³⁹ The occupation of several of the promoter sites is abnormal in T cells from patients with SLE. NF- κ B nuclear activity has been shown to be diminished, and the artificial replenishment of the p65 subunit increases IL2 production in these cells.^{40–41}

Another position that has been shown to be an abnormally regulated site in SLE T cells is the –180 position. It comprises a binding site for CREB/CREM. When CREB is phosphorylated (pCREB), it acts as a positive factor, greatly enhancing transcription. On the other hand, CREM displaces pCREB and thus acts as a transcriptional repressor.⁴² It has been proposed that the balance between these two factors is extremely important for the regulation of IL2 transcription.⁴³

A consistent finding in the IL2 promoter of T cells obtained from patients with lupus has been an imbalance in the CREM/CREB ratio found at the –180 site. It represents the fact that T cells from patients with lupus exhibit abnormally high levels of the transcriptional repressor CREM. This abnormality has been directly linked to the characteristic IL2 production defect of lupus T cells.⁴⁴ In fact, it is probably the final common pathway for several of the defects that have been associated with the IL2 production defect of the lupus T cell (fig 1).

CREM inactivation by an antisense CREM plasmid is capable of correcting the abnormally increased CREM binding present in lupus T cells and, by doing so, it directly mitigates the hampered IL2 secretion.⁴⁵

Numerous factors have been detected in T cells derived from patients with SLE that directly or indirectly affect the balance between CREB and CREM. Interestingly, some of these aberrations appear to be intrinsic to the T cell, whereas others reside in the sera of lupus patients. A mechanism by which SLE serum is able to inhibit IL2 transcription is found in its capacity of activating a kinase called Ca²⁺/calmodulin-dependent kinase IV (CaMKIV).⁴⁶ We have reported evident alterations in the levels and intracellular compartmentalisation of this kinase. Lupus T cells have more CaMKIV and it has been demonstrated that such alteration is a direct consequence of factors present in the sera of patients with lupus, particularly those in which anti-CD3/TCR activity is detected.⁴⁶ By a still unknown mechanism, lupus-derived sera produces migration of CaMKIV into the nuclei of normal T cells. In the nucleus, this kinase is able to activate CREM and increase its binding to the –180 site. This leads to a reduced pCREB/CREM ratio and to a reduction in IL2 transcription. As expected, lupus T cells exhibit increased amounts of CaMKIV in the nuclei. The importance of this phenomenon is highlighted by the fact that inhibition of CaMKIV activity by overexpression of a dominant negative CaMKIV isoform abolishes the IL2 inhibiting effect of SLE sera.⁴⁶

As expected, CREB and CREM are involved in the regulation of other genes and changes in their levels alter the expression of several proteins apart from IL2; *c-fos*, which assembles with *jun* to form AP-1, is one of the genes whose transcription is hampered by excessive amounts of CREM. Thus, although alterations of the CREB/CREM ratio probably lead to skewed transcription of a large number of genes, IL2 transcription is

Figure 3 Several defects contribute to the lipid raft pre-clustering and abnormal T cell activation process in SLE T cells. Many of the molecules involved represent molecular targets; the suppression of those shown in red and the replenishment of the ones depicted in blue associate with the correction of the lupus phenotype.

affected by at least two distinct mechanisms: the altered occupation of the –180 site, and decreased AP-1 site occupation (fig 1).⁴⁵

PP2A (protein phosphatase 2A) is a highly conserved enzyme present in virtually all eukaryotic cells.⁴⁷ It is the principal enzyme responsible for the dephosphorylation (and thus inactivation) of CREB in T lymphocytes, and has been shown to be able to suppress IL2 production.^{48–49} PP2A levels, measured as protein and mRNA, are abnormally elevated in T cells from patients with SLE.⁵⁰ PP2A activity is also augmented in lupus T cells and contributes to the defect in IL2 production by altering the pCREB/CREM ratio. Inhibition of PP2A in lupus T cells (by siRNA or by the expression of dominant negative isoforms) increases pCREB binding to the promoters of *c-fos* and IL2 and, by doing so, corrects the IL2 production defect (fig 2).⁵⁰

Thus, T cells from patients with SLE have a number of alterations which, through different mechanisms, result in limited ability to produce IL2. Some of the defects result in increased CREM levels, whereas others result in decreased CREB phosphorylation and thus its capacity to stimulate gene transcription.⁵¹

MOLECULAR TARGETS

According to what has been outlined before, several molecules have arisen in the last few years that represent plausible therapeutic targets (figs 3, 4). The studies that have been described predict that the inhibition or replenishment of certain pivotal molecules will lead to the correction of the bizarre behaviour that the lupus T cells exhibit during *in vitro* analyses.

The pathological mechanisms that have been described imply that the phenotypic T cell defects are susceptible to being corrected by the alteration of the expression levels and function of more than one molecule. Thus, the correction of the aberrant T cell activation process is theoretically possible by the inhibition of caspase-3, CD44, Rho kinase (ROCK), FcRγ and Syk; the former would have similar effects to achieving an increased expression of CD3ζ chain (fig 3). Some of these

Figure 4 T cell intrinsic defects as well as factors present in lupus sera contribute to the defective IL2 production characteristic of SLE T cells. The expression of NF-κB subunits, as well as the inhibition of PP2A and CREM, lead to the correction of the IL2 production defect in T cells obtained from patients with lupus.

interventions would probably improve the IL2 production defect, but the inhibition of PP2A and CREM would almost certainly accomplish it (fig 4).

As indicated before, the prevalence of the described defects is not homogeneous within the population of patients with SLE. Thus, each individual patient will probably exhibit a distinct combination of abnormalities that will define which interventions are more adequate in that particular case. Furthermore, it will be necessary to identify the relationship between the different phenotypic aberrations and discrete clinical syndromes. A straightforward liaison is predicted to exist between increased expression of CD44, increased phosphorylation of ERM, and T cell infiltration and subsequent kidney inflammation.³¹

Obviously, an important issue is whether the correction of these deficiencies will translate into clinical improvement. The experimental data strongly suggest that targeting of these abnormally expressed molecules will result in improvement of the involved pathogenic mechanisms which in turn will prove to be of great clinical importance. This concept, however, is yet to be proven and its significance will have to be considered within clinical practice.

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