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Immune Cell Signaling Aberrations in Human Lupus

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Abstract

A large array of heterogeneous aberrations of the immune system have been described in systemic lupus erythematosus (SLE). Since the function and the fate of the immune system cells are governed principally by the biochemical events that follow ligation of specialized cell-surface receptors, we will review in this article recent developments in our understanding of abnormalities in the biochemistry of signals generated either by the antigen–receptor complex or by systems of costimulatory cell-surface molecules, like the CD28/CTLA4:CD80/CD86 and the CD40:CD40L pairs found on the surface membrane of lupus immune cells.

Key Words

Systemic lupus erythematosus
Human autoimmunity
Lymphocytes
Signal transduction
Antigen receptor
Costimulation

Introduction

The etiopathogenesis of systemic lupus erythematosus (SLE) remains unknown. It is thought that a dominant role for the expression of the disease is played by a dysregulated immune system. Numerous abnormalities concerning the function of the immune cells have been characterized *(1,2)*. The immune system consists of cells performing specialized functions under a strict regulatory control. Most aspects of lymphocyte function are determined by intracellular signals that are generated when highly specialized cell-surface molecules encounter their specific ligands. The most prominent example is the antigen receptor (AgR) found on the surface of T cells (TCR) and B cells (BCR). When the AgR becomes ligated by its specific antigen (Ag) or anti-receptor monoclonal antibody (MAb), a well-regulated cascade of biochemical events ensues *(3,4)*. This cascade in fact consists of several different, but inter-

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related biochemical pathways, acting in a concerted fashion. Other cell-surface molecules (linked or not) with the AgR may participate in signaling when simultaneously bound to their ligands or counterreceptors, giving rise to additional biochemical events in the cytoplasm. These complementary signals can enhance, suppress, or redirect the biochemical pathway initiated after TCR or BCR ligation, depending on the nature of the participating surface molecules. The participation of costimulatory signals in the AgR pathway substantially amplifies the initially produced response, whereas the participation of counterstimulatory molecules diminishes or redirects the signaling cascade. Binding of Ag or anti-receptor MAbs to the AgR eventually cause cell activation, proliferation, production and release of cytokines and other soluble mediators, or can cause inhibition of cell growth, anergy, and apoptosis. It is thus not only the function, but the fate also of the immune cell that are determined by the particular biochemical events that ensue following AgR ligation. Which of the above different directions the cell will follow depends at least in part on the participation or not of specialized cell-surface molecules, either coreceptors or other signal-related receptors. These receptors may dictate which intracellular biochemical pathway will be preferentially used and which will be avoided. Since the signaling process initiated by the AgR is important for lymphocytes, it is reasonable to ask whether this aspect of immune cell function is disturbed in lupus. Indeed, the spectrum of abnormalities in immune regulation and the heterogeneous immune cell dysfunction in SLE may have a common denominator, and cell signaling can serve as a plausible candidate.

AgR-Mediated Signaling Aberrations in Lupus Lymphocytes

TCR and BCR are two members of the immunoglobulin superfamily of cell-surface receptors. They are hetero-oligomeric protein complexes constructed in such a way that gives rise to a bifunctional molecule. The highly polymorphic part of the AgR is the surface immunoglobulin (sIg) for B cells and the TCRα/β (or TCRγ/δ) heterodimer in T cells, and it is this part that serves as the antigen-recognition and antigen-binding structure. The rest of the AgR is invariant and serves as the signal-generating module. In T cells, the invariant complex is made of the CD3γ–, δ–, ε-chains and the TCRζ-chain homodimer (or TCR$\zeta\eta$ heterodimer in a minority of cells), whereas in B cells, the signal transducer is the Ig-α/Ig-β heterodimer *(3–5)*. The precise stoichiometric composition of the AgR complex though remains uncertain, despite considerable research efforts. When this surface AgR becomes ligated by either its specific Ag or by anti-AgR MAb (i.e., an anti-CD3 MAb for T cells or an anti-Ig for B cells), the cytoplasmic tails of the AgR-invariant chains become rapidly and transiently tyrosyl-phosphorylated. Phosphorylated tyrosines are found in the context of a conserved motif called immunoreceptor tyrosine-based activation motif (ITAM), which represents two copies of the sequence YxxL/I interrupted by a six to eight amino acid stretch. This motif consists of the signaling module of all the cellular proteins that possess it *(6)*. Although ITAMs are anchoring sites for src-homology 2- (SH2) domain-containing proteins, it is the precise amino acid sequence of the different ITAMs that dictates which specific SH2-domain containing protein(s) will dock on it. Even though the exact sequence of events is still unknown, it is proposed that the tyrosyl phosphorylated ITAMs of the AgR complex become anchoring sites for the SH2 domains of Syk/ZAP-70 family of protein tyrosine kinases (PTK), which in turn become tyrosine phosphorylated by the action of another family of signaling molecules the src family of

PTK. Different members of the src and the Syk-family of PTK are expressed differentially in T and B cells, and different SH2-containing proteins attach to different ITAMs. However, the biochemical cascade of signaling events is similar in lymphocytes. Activation of src- and syk-PTKs, is a key event for further signal propagation. Under the influence of activated PTKs a number of different downstream mediators become tyrosine-phosphorylated and activated, the final result being the activation of transcription factors, which in turn mediate the transcription of specific target genes. A well-studied paradigm is the pathway initiated by the tyrosine phosphorylation (and activation) of the isozymes of phospholipase C (PLCγ1 isozyme predominating in T cells and PLCγ2 in B cells) *(7)*. Activated PLCγ translocates to the cell membrane where it cleaves the membrane phosphatidyl inositol 4,5-bis-phosphate producing diacylglycerol (DAG) and inositol trisphosphate (IP3) *(8)*. The former of these second messengers activates protein kinase C (PKC), and the latter binds on its receptor causing the release of intracellularly stored free Ca^{2+} *(9,10)*. The increase in intracellular Ca^{2+} concentration activates calcineurin and the Ca^{2+}/calmodulin-dependent kinase II. Calcineurin (a serine/threonine phosphatase inhibited by cyclosporin A) in turn dephosphorylates the cytoplasmic form of transcription factor NF-AT, which then translocates to the nucleus in its active state mediating (along with other transcription factors) the transcription of target genes, among them being the gene for IL-2, since NF-AT binds to the distal promoter of IL-2. The increase of intracellular Ca^{2+} concentration, therefore, consists of one pivotal step in the AgR signal transduction pathway. The magnitude of the Ca^{2+} response has been correlated with the affinity of the receptor for different ligands *(11)*, and the functional consequences of this response are reflected on the outcome for the cell, i.e., activation or anergy *(12)*. The study of AgR-mediated Ca^{2+} fluxes then consists of a useful tool for evaluating the AgR signaling cascade. This tool was chosen to evaluate the AgR signal transduction events for T and B cells in SLE.

TCR-Mediated Signaling in Lupus

The TCR/CD3 complex-mediated Ca^{2+} responses were analyzed in 21 lupus patients, and compared to the responses of normal volunteers and of patients with other systemic autoimmune rheumatic diseases. It was demonstrated that two different anti-CD3 MAbs used to elicit Ca^{2+} responses produced significantly higher Ca^{2+} fluxes in fresh peripheral unmanipulated lupus T cells as well as in short-term lupus T cell lines, compared to the two different control populations *(13)*. The two major T cell subpopulations, $CD4^+$ and $CD8^+$ T cells, responded similarly. The release of intracellularly stored Ca^{2+} contributed significantly to this effect, but the production of IP3, the major mediator of intracellular Ca^{2+} release, was only slightly elevated in lupus T cells, suggesting either a hypersensitive IP3 receptor, or the dominant contribution of IP3-independent Ca^{2+} release pathways, which are incompletely understood yet.

To address the question of possible influences on signaling of cytophilic proteins or of a state of preactivation of circulating lupus T cells, IL-2 dependent short-term T cell lines were established and studied as well. Lupus, but not control T cell lines again displayed significantly increased TCR/CD3-mediated signaling events, consisting of significantly higher Ca^{2+} responses, and higher levels of IP3 production. The differences obtained from fresh peripheral lupus T cells and lupus T cell lines were disease-specific and disease activity-independent, suggesting that they may represent intrinsic defects of the lupus T cell signaling machinery.

To further characterize the TCR/CD3-initiated signaling defects, we studied 22 patients with SLE, 12 patients with other systemic rheumatic diseases, and 14 normal donors. The early (1 min) TCR/CD3-mediated tyrosine phosphorylation of cellular proteins with a molecular size between 36 and 64 kDa was increased in 15 of 21 SLE patients, compared to normal or disease control subjects. Immunoblots using anti-ζ antibodies and flow cytometric studies using permeabilized cells showed that 10 of 22 lupus patients tested lacked the expression of TCRζ. Using Northern blots and reverse transcriptase-polymerase chain reaction we found that the TCRζ mRNA was missing in 3, decreased in 3, and apparently normal in 2 patients, but was always present in control subjects. These studies strongly suggest that TCRζ chain expression is either decreased or absent in the majority of patients with SLE, but not in patients with other systemic rheumatic diseases, regardless of disease activity, treatment status, or clinical manifestations. The deficient TCRζ expression may represent intrinsic defects modulating lupus T cell function *(13a)*.

A biochemical defect described earlier in lupus T cells, the decreased activity of the cAMP-dependent protein kinase A- (PKA) isozyme I may offer an explanation for these supraphysiological TCR-mediated Ca^{2+} responses. Activation of this serine/threonine kinase normally causes a termination of the TCR-induced Ca^{2+} fluxes, possibly through downregulating surface TCR, and also by decreasing the sensitivity of the IP3 receptor. The activity of this enzyme is decreased in lupus T cells, and this deficiency of inhibition may participate in the pathophysiology of increased TCR/CD3-mediated Ca^{2+} responses (Fig. 1) *(14)*.

Abnormalities of PKC function (also a serine/threonine phosphatase) have also been reported. Using the PKC agonist phorbol myristate acetate (PMA), it was found that early events in the signaling cascade were disturbed in lupus T cells. Specifically, the PMA-induced phosphorylation of a 80-kDa substrate was decreased, and the expression of the activation molecule CD69 on the cell surface, as well as the increase of the intracellular pH were reduced *(15,16)*. It is thus possible that the AgR-initiated signaling cascade in lupus T cells suffers from multiple defects at different downstream points.

BCR-Mediated Signaling in Lupus

Another study analyzed the AgR-mediated responses in fresh peripheral unmanipulated lupus B cells. The sIgM- and sIgD-initiated Ca^{2+} fluxes were studied in 21 SLE patients using an anti-IgM $F(ab')_2$ fragment and two different anti-IgD MAbs. The Ca^{2+} responses of the patients with SLE were compared with those of patients with other systemic rheumatic diseases or of normal individuals. These two different modes of BCR stimulation produced significantly increased Ca^{2+} responses in the B cells of lupus patients compared to the normal- or the disease-control population studied *(17)*. Again, as in lupus T cells, the intracellularly stored Ca^{2+} contributed significantly to these higher responses, and likewise, the production of IP3 was only slightly elevated in lupus B cells compared to their normal counterparts. Since the earliest known event in AgR signaling is the activation of PTK and the production of tyrosine phosphorylated proteins, this step was also addressed in lupus B cells. It was found that the profile of tyrosine-phosphorylated proteins was increased for lupus B cells. More specifically, at least four protein substrates with molecular sizes between 36- and 64-kDa were obviously tyrosine hyperphosphorylated after ligation of the sIgM. This increased production of phosphotyrosines correlated with increased Ca^{2+} fluxes (Fig. 1).

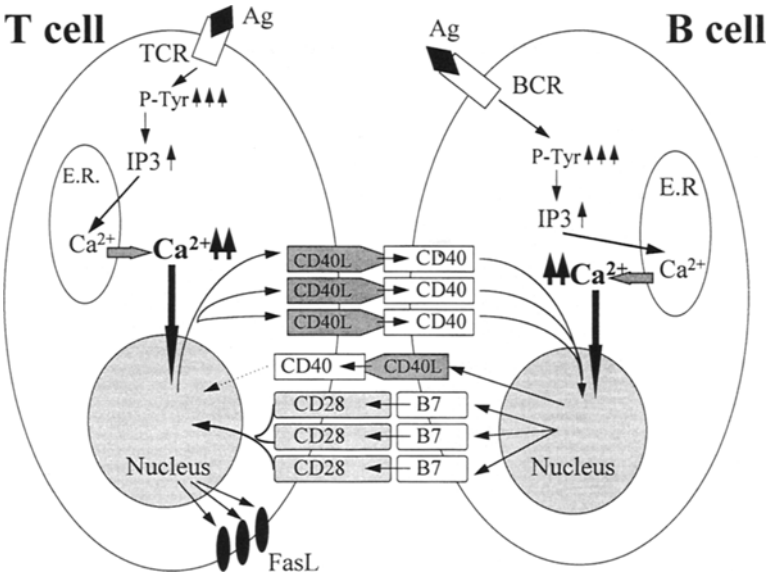

Fig. 1. Outline of signal transduction aberrations encountered in lupus lymphocytes. AgR ligation in B cells (BCR) and in T cells (TCR) leads to an early supraphysiological increase of tyrosine phosphorylated cellular proteins in B as well as in T cells. This is followed by increased production of inositol 1,4,5-trisphosphate and increased release of Ca^{2+}. Cytoplasmic Ca^{2+} is a second messenger mediating the activation of enzymes and the transcription of several different genes. The cell-surface expression of CD40L and FasL (transcription of both markers is Ca^{2+}-dependent) is significantly increased on lupus T cells. Resting and activated lupus B cells also hyper-express functional surface CD40L. The induction of CD80 costimulatory molecule is defective in APCs (B- and non-B APCs), and the function of the CD80-mediated pathway is decreased in lupus. The expression of CD86 though has been reported to be increased in lupus B cells. The hypersensitive AgR-mediated signaling may result in altered lymphocytic responses in lupus and the aberrant expression of costimulatory molecules may modify the cognate and helper interactions between autoreactive T and B cells in patients with systemic lupus erythematosus. Ag; Antigen, P-Tyr; tyrosine-phosphorylated proteins, E.R.; endoplasmic reticulum.

The pathophysiology of the supranormal lupus B cell responses is unknown. The function of B cell coreceptors that positively (CD19) or negatively (CD22, FcγRIIB) regulate BCR-generated signals should be addressed. CD19 coligation with BCR causes an exponential increase in the magnitude of the BCR signals *(18)*. CD19 is found in association with CD21 and CD81, comprising the complement receptor type 2 (CR2) complex. The expression of CR2 on the surface of lupus B cells is decreased, and therefore, CD19 may not be a good candidate to explain the high BCR-mediated responses. The decrease of CR2 expression was initially claimed as a potentially intrinsic lupus B cell defect *(19)*. Nevertheless, levels of CR2 were later reported to correlate inversely with disease activity, thus favoring the view of an acquired phenotype *(20)*.

The signaling inhibitors may be better candidates for study but the expression and function of the two known B cell signaling downregulators is unknown in SLE. These surface molecules possess an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic tails, and exert their signal-inhibitory actions on phosphorylation of the ITIM tyrosine residue via the ITIM-mediated recruitment of SH2-containing phosphatases

SHP-1 and SHIP *(21,22)*. Data obtained from the study of animal models make ITIM-bearing and ITIM binding molecules attractive candidates for study in human lupus. CD22-knockout mice display enhanced anti-sIgM-initiated Ca^{2+} responses and autoantibody production, and the SHP-1-deficient animals (*motheaten* mice) behave likewise *(23–25)*. FcγRIIB-deficient mice have increased serum immunoglobulin levels *(26)*, an abnormality very characteristic, nevertheless nonspecific, for human lupus, too. Abnormalities in the family of Fc receptors have also been identified in SLE. Extracellular domain polymorphisms of the FcγRIIA molecules (also known to bear an ITAM module in the cytoplasmic tail), which can have a functional impact for the clearance of immune complexes, have been described in lupus patients, and recently other polymorphisms were also reported to affect FcγRIIIA receptors *(27,28)*. Nevertheless, there are no direct studies in lupus patients addressing the molecular composition, expression, or function of the FcγRIIB, the only Fcγ receptor that is present on the surface of B cells.

These data challenge therefore the view that B cell hyperactivity in lupus is a product of abnormalities in the T cell-compartment function. The abnormally high signaling events obtained for lupus B cells were disease-specific and disease activity-independent, pointing toward an intrinsic defect in lupus B cells, which may possibly contribute to the pathogenesis of SLE.

It was thus shown that lupus lymphocytes display very similar and abnormally high TCR- and BCR-mediated early signal transduction events consisting principally of elevated Ca^{2+} fluxes in a disease-specific fashion. If these aberrant responses are of functional importance for the immune cell, it should be reflected on Ca^{2+}-dependent biochemical processes. Indeed, abnormalities in TCR-mediated Ca^{2+}-dependent events have been described for SLE lymphocytes. The expression of two Ca^{2+}-dependent T cell-surface molecules of paramount biological importance, CD40L (discussed below) and FasL *(29)*, is abnormally high in T cells from patients with SLE *(30)*. For the latter, the TCR/CD3-induced upregulation of FasL was significantly higher for activated lupus T cells compared to activated T cells obtained from normal volunteers and patients with rheumatoid arthritis. Increased FasL on lupus T cell was functional as evidenced by the enhanced killing of an Fas-bearing normal T cell line. On the other hand, the production of IL-2, which is also partly Ca^{2+}-dependent, is lower for lupus T cells *(31)*, indicating that for IL-2, other transcriptional pathways may prevail or at least be equally important to the Ca^{2+}-dependent pathway. Even though the exact biological significance of supranormal AgR-mediated early signaling events is not well understood, this abnormality may represent a common background for the different and even contrasting aberrations described in the past regarding the regulation and function of lupus lymphocytes.

CD40:CD40L (CD154) Interaction in Lupus

An important costimulatory signal is generated from the interaction of the constitutively expressed CD40 molecule on the surface of B cells (and other Ag-presenting cell [APC] types) with its inducible ligand CD40L (CD154). When B cells present Ag to the TCR of T-helper cells in the context of MHC Class II molecules, the T cell becomes activated. An early T cell-activation event is the induction of CD40L expression on the T cell surface *(32)*. The essential signal for the expression of CD40L is an increase in the concentration of free intracytoplasmic Ca^{2+} *(33)*. CD40L is transiently, but intensely upregulated on the T cell surface, and it mediates the generation

of a CD40-initiated stimulus in the B cell. This CD40-mediated signal is important for B cell activation, upregulation of B7-1 (CD80) and B7-2 (CD86) on the surface of resting B cells, proliferation, and immunoglobulin isotype switching *(34)*. Precisely how these events take place at the molecular level is not yet clear, but it was recently shown that although BCR-mediated signaling involves the preferential engagement of the ERK signaling pathway and only the weak involvement of the JNK and the p38 kinase pathways, CD40-initiated signals involve the intense activation of the JNK and p38 kinases pathways *(35–37)*. These biochemical differences may represent the molecular basis for the different phenotypic and functional outcomes in B cells stimulated via the BCR only, or via BCR and CD40.

The important functions executed by CD40: CD40L molecules during thymus-dependent immune responses led to the evaluation of its function in lupus, where autoantibody production is generally considered a T cell-dependent B cell response. First, although the baseline expression of CD40L on circulating lupus T cells was found to be increased only in some SLE patients, the activation-mediated CD40L upregulation was not only significantly enhanced, but prolonged as well *(38)*. This abnormally regulated CD40L was shown to be functional, since it induced the expression of B7 on the surface of B cells. Because the expression of CD40L on T cells is TCR-mediated, and since it is Ca^{2+}-dependent *(33)*, it could be claimed that the increased TCR-initiated signaling events encountered in lupus T cells correlate to the enhanced CD40L upregulation (Fig. 1). These findings regarding the enhanced expression of CD40L on lupus T cells were confirmed by another group of investigators who extended the study of CD40L expression to lupus B cells. Normal B cells do not physiologically express CD40L, but after stimulation with pharmacological doses of a combination of chemical mediators, they can only weakly express CD40L on their surface *(39)*. Circulating lupus B cells on the contrary either express CD40L or were induced to express it at high levels, being intensely (T cell-like) positive. The source of this aberrantly expressed marker was indeed the B cell, since increased levels of CD40L mRNA were detected in the cytoplasm of lupus B cells *(40)*. CD40L on B cells represented a functional molecule as was shown by the blocking effects of an anti-CD40L MAb on the production of autoantibodies by these B cells. Since activated T cells can also express CD40 on their surfaces *(41)*, it was postulated that the CD40:CD40L pathway can participate not only in autonomous lupus B–B cell interactions, but in bidirectional interactions involving T and B cells in SLE as well.

The function of the CD40:CD40L pair is of upmost importance for thymus-dependent immune responses. It is essential not only for cognate T–B cell interactions, but it provides the B cell with an important second signal leading to the upregulation of B7 surface molecules. This in turn will provide the T cell with a crucial CD28-mediated anergy-avoiding second signal, potentially by coupling the TCR pathway with the activation of the JNK pathway *(42)*. Considering the CD40:CD40L pair a good candidate for immune intervention in the autoimmune diseases, several groups administered blocking agent(s) to interfere with its function. The effect of these blocking agents in different murine autoimmune models was encouraging *(43–45)*. Administering an anti-CD40L MAb to lupus-prone mice completely prevented the development of lupus glomerulonephritis, even when only a single dose of the blocking MAb was administered *(46)*. Importantly also, generalized immunosuppression did not ensu and autoantibodies were not produced in the anti-CD40L MAb-treated mice. These data not

only further establish the biological significance of CD40:CD40L interaction, but they also point toward the potential usefulness of similar immune interventions in SLE.

CD28/CTLA4:CD80/CD86 Interaction in Lupus

In addition to the specific TCR/CD3-mediated signal, at least one costimulatory signal provided by an APC is required for the initiation of an effective T cell response, as well as for its maintenance and/or downregulation. A major T cell costimulatory pathway involves interactions between the CD28 and CTLA4 molecules on T cells and their counter-receptors, CD80 (B7-1) and CD86 (B7-2) molecules on APCs. CD28 is constitutively expressed on the majority of resting $CD4^+$ T cells, on half of the resting $CD8^+$ T cells, and on certain NK cells in the peripheral blood, and its expression increases following activation. CTLA4 is expressed only on activated T cells. CD86 is constitutively expressed on resting peripheral monocytes and dendritic cells, whereas expression of CD80 at substantial levels on these cells is primarily activation-induced. Expression of both molecules on all other APCs requires stimulation. CD28- and CTLA4-mediated signals have distinct effects on T cells by integrating additional biochemical parameters in the TCR-signaling pathway *(42)*. Ligation of CD28 following a TCR-mediated signal results in secretion of cytokines, upregulation of CTLA4 mRNA, T cell proliferation, and differentiation. The absence of CD28-mediated signals results either in impaired cytotoxic responses and/or in a long-lasting anergic state. In contrast, CTLA4 delivers a downregulatory signal to T cells. It may also mediate the deletion in the periphery of autoreactive T cells that have escaped previous deletion in the thymus *(47,48)*.

Recent studies have examined CD28/CTLA4:CD80/CD86 interactions in patients with SLE, but exactly how these signaling pathways are potentially involved in the pathogenesis of the disease remains obscure *(49)*. In patients with either active or inactive disease, the mean percentages of $CD28^+$ peripheral blood T cells of both $CD4^+$ and $CD8^+$ subsets are decreased *(50,51)*, although with a remarkable variation in individual SLE patients (range 1–50%), in contrast to normals (range 1–15%) *(51)*. Circulating $CD28^-$ T cells in SLE patients are significantly increased *(51)*. Lymphocytes from patients with active disease show increased CD28 mRNA compared to normal subjects *(50)*. Anti-CD3-induced apoptotic death of $CD28^+$ T cells is significantly accelerated in vitro in SLE providing a possible explanation for the loss of these cells from the peripheral blood in vivo, whereas apoptosis of $CD28^-$ T cells is hardly detected either in patients with SLE or in normal persons *(51)*. Peripheral $CD28^+$ T cells from patients with SLE exhibit normal proliferative responses in vitro to both CD28-independent and CD28-dependent stimuli, using either an anti-CD28 MAb *(51)* or stable transfectants expressing the CD80 (BB1) molecule *(52)*. These results suggest that although CD28-mediated signaling in peripheral blood T cells is not intrinsically impaired, expression of CD28 may be relevant to aberrant T cell apoptosis seen in these patients. Alternatively, the decrease of $CD28^+$ T cells in the periphery may result from selective migration of these cells in sites of autoimmune injury, as was shown in inflammatory infiltrates from rheumatoid arthritis synovial effusions *(53)*. Expression of CTLA4 has not been demonstrated in vivo on peripheral T cells from patients with SLE. In vitro, substantial expression of CTLA4 in SLE T cells was detectable at 5 d after stimulation; kinetics of CTLA4 induction were similar between patients with SLE and healthy individuals *(51)*. Since expression of CD80 molecules on activated

Table 1. CD28, CTLA4, CD80, and CD86 in SLE

Molecule	Cell type	Comment
CD28	T cells, $CD4^+$	Decreased expression, retains function
	T cells, $CD8^+$	Decreased expression, retains function
CTLA4	T cells	Not expressed at detectable levels in vivo, normal kinetics after induction in vitro
CD80	Activated B cells	Increased expression
	Resting B cells	Normal expression
	Peripheral MNCs	Impaired upregulation after induction in vitro
	Dermal APCs	Increased expression
	Keratinocytes	Increased expression
CD86	Activated B cells	Increased expression
	Resting B cells	Increased expression
	Dermal APCs	Increased expression

T cells, acting also as APCs, prevents the generation of cytotoxic T cell responses in vitro *(54)*, increased interaction between CD80 and CD28 in vivo may correlate with deficient deletion of autoreactive T cells in lupus patients. Folzenlogen et al. examined the expression of CD80 and CD86 on the cell surface of peripheral blood B cells obtained from normal persons and patients with allergy and SLE. This group reported a 7- and a 2.5-fold increase of CD86 expression on resting and activated B cells, respectively, from patients with SLE compared to normals. CD80 is also significantly overexpressed in activated, but not in resting B cells from patients with SLE, though at lower than CD86 levels *(55)*. Another study examined the role of B cells as auto-APCs in activating autoreactive T cell responses, and demonstrated that expression of CD80 and/or CD86 molecules by B cells is a prerequisite for breaking T cell tolerance to self-antigen *(56)*. Therefore, overexpression of the costimulatory molecules on circulating B cells in patients with SLE may play a major role in the continuous autoreactive T cell help to lupus B cells leading to the production of autoantigens.

On the other hand, non-B APCs from patients with SLE, but not from normal persons, fail to upregulate the in vitro surface expression of CD80 following stimulation with interferon-γ in a disease-independent fashion. Replenishment of functional CD80 molecule in the culture environment significantly increased the responses of SLE T cells to tetanus toxoid and to an anti-CD3 MAb *(57)*. Similarly, the decreased responses of lupus T cells to anti-CD2 *(58)* are reversed in the presence of adequate CD28-mediated stimuli provided either by B cells from normal donors or by an anti-CD28 MAb *(59)*. In lesional skin of patients with SLE and discoid lupus, but not in normal skin, an abundance of CD80 and CD86 expressing cells in the dermis and epidermis has been observed *(60,61)*, whereas the majority of infiltrating T cells are $CD28^+$ *(61)*.

Taken together the above findings suggest that aberrant expression of CD28, CTLA4, CD80, and CD86 molecules on T cells and APCs at different disease stages in patients with SLE may contribute to pathology (Table 1). It can be hypothesized that increased numbers of autoreactive T and B lymphocytes escape peripheral deletion, because the necessary

costimulatory signal provided at sites of autoimmune injury is inadequate. Alternatively, T cells may ignore the autoantigen in an inappropriate milieu. Other findings are more compatible with the notion that increased costimulation is responsible for the cognate interactions between T cells and APCs required for production of pathogenic autoantibodies. According to the proposed strength-of-signal-model, CD28 costimulation can have distinct effects on the immune response depending on Ag dose, APC function, cytokine milieu, and level of costimulation *(47)*. Low levels of costimulation at early time-points may help the progression of the autoimmune response, whereas high levels of costimulation coupled with high TCR occupancy may in fact downregulate the response, providing protection from injury. This effect, caused by extensive TCR-mediated activation plus CD28 costimulation, leads to activation-induced cell death and explains the so-called "high zone tolerance" or "clonal exhaustion." In addition, hyperstimulation may lead to upregulation of CTLA4 on activated autoreactive T cells resulting also in "protective" inhibition of their function *(47)*. Interrupting the crosstalk of CD28 with CD80 and CD86 may be of clinical value. To test this hypothesis, Finck et al., treated lupus-prone NZB/NZW mice with CTLA4-Ig, a soluble recombinant fusion protein that blocks the engagement of CD28 by CD80 and CD86. CTLA4-Ig treatment blocked autoantibody production and prolonged the survival of mice, even when it was administered late, during the most advanced stage of clinical illness *(62)*. Nevertheless, additional studies are necessary to understand whether disruption of CD28-mediated pathways may clinically benefit patients with SLE.

Relevant Data from Lupus-Prone Animal Models

The contribution of genetic factors to the development of autoimmunity is unequivocal. Exciting data have been reported lately from the genetic analyses of lupus-prone murine strains *(63)*. The study of strains stemming out of the prototype New Zealand Black/New Zealand White (NZB × NZW) lupus model using the newer and revolutionary microsatellite-based genetic techniques has identified several loci which are correlated either with predisposition for the disease or with specific clinical manifestations. Different groups have produced remarkably similar results, and some loci found during different genome-wide searches may be identical, or near-identical, despite the different markers used, strains studied, and even names these loci were given. Much attention was drawn to a segment found in murine chromosome 4, termed *nba-1*, *Sle2*, or *Lbw-2/Sbw-2* by three different groups *(64–66)*. All three loci mapped in a 9cM interval, thus making it possible that they localize into a single gene. To study its contribution to lupus, the *Sle2* segment was expressed separately in a lupus-resistant genetic background. *Sle2* produced a picture attributable to B cell hyperactivity only, with the production of autoantibodies and B cell hyperresponsiveness to BCR-delivered stimulation *(67)*. *Sle2* was nonetheless not able to produce the full-blown murine syndrome, as were other separately expressed lupus-related genetic segments similarly unable to reproduce fully lupus *(68)*. Although studies addressing directly any signaling abnormalities in murine lupus are missing, the more direct and promising genetic studies have revealed at least one candidate locus that contributes phenotypically to lymphocytic hyperresponsiveness in lupus animal models.

The extent to which these data apply to the human disease is unknown, but the impact of genetic factors on the expression of human SLE is well accepted. Recently, a study of lupus families using microsatellite markers revealed

a strong linkage with a locus mapped at chromosome region 1q41-42 *(69)*. The detailed genetic composition of this segment, its functional products, and its relationship (if any) with constituents affecting the function of lupus lymphocytes are not yet known.

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