Differential Expression and Molecular Associations of Syk in Systemic Lupus Erythematosus T Cells


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Differential Expression and Molecular Associations of Syk in Systemic Lupus Erythematosus T Cells

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Diminished expression of TCR ζ and reciprocal up-regulation and association of FcRγ with the TCR/CD3 complex is a hallmark of systemic lupus erythematosus (SLE) T cells. In this study we explored whether differential molecular associations of the spleen tyrosine kinase Syk that preferentially binds to FcRγ contribute to pathological amplification of signals downstream of this “rewired TCR” in SLE. We detected higher amounts of Syk expression and activity in SLE compared with normal T cells. Selective inhibition of the activity of Syk reduced the strength of TCR-induced calcium responses and slowed the rapid kinetics of actin polymerization exclusively in SLE T cells. Syk and ZAP-70 also associated differently with key molecules involved in cytoskeletal and calcium signaling in SLE T cells. Thus, while Vav-1 and LAT preferentially bound to Syk, phospholipase C-γ1 bound to both Syk and ZAP-70. Our results show that differential associations of Syk family kinases contribute to the enhanced TCR-induced signaling responses in SLE T cells. Thus, we propose molecular targeting of Syk as a measure to control abnormal T cell responses in SLE. The Journal of Immunology, 2008, 181: 8145–8152.

S

gnal transduction through the TCR has been shown to be augmented in T cells derived from patients with systemic lupus erythematosus (SLE)1 (1, 2). Specifically, the kinetics of TCR-induced intracellular tyrosine phosphorylation, polymerization of actin, and intracellular calcium flux are accelerated in SLE compared with normal T cells (3, 4). Recent studies have identified the contribution of the altered structure of TCR in mediating abnormal T cell responses. In a majority of patients with SLE T cells, TCR ζ-chain undergoes extensive down-regulation and, instead, reciprocal up-regulation and association of a homologous FcR γ-chain with the TCR is observed (3, 5). Signaling through this “rewired TCR” is presumed to be stronger because of the association of FcRγ with the spleen tyrosine kinase Syk, which is 100 times enzymatically more potent than ZAP-70, the kinase that is predominant in normal T cells and traditionally associates with TCR ζ (6, 7).

Differences in the molecular associations and activities of Syk and ZAP-70 have been reported and have been ascribed to the differences in the structures of these two kinases (8, 9). The recruitment for association of ZAP-70, but not Syk, with Src kinase proteins for enzymatic activation is one such example (8, 9). Similarly, differential expression of ZAP-70 and Syk in T cell subsets might explain differential functional outcomes of TCR signaling. For example, in vitro activated effector CD4 T cells express high amounts of Syk, and in these cells Syk also demonstrates greater activities of these kinases in abnormally regulating these events. Furthermore, human ZAP-70−/− T cells that express Syk also produce lower amounts of IL-2 compared with freshly activated naive T cells. Similarly, human ZAP-70−/− T cells that express Syk also produce lower amounts of IL-2 compared with T cells that express ZAP-70 (11). These observations suggest a strong role for Syk/ZAP-70 kinases in shaping differential functional outcomes of TCR signaling.

However, how Syk contributes to the augmentation of TCR-induced signaling in SLE remains unknown. In this study, we hypothesized that Syk and ZAP-70 associate with disparate signaling molecules in normal and SLE T cells and contribute to the hyperexcitable phenotype of SLE T cells. Our assumption was based on several observations. First, the binding between Syk and FcRγ was observed in SLE but not normal T cells (5). Second, Syk but not ZAP-70 was found to be associated with lipid rafts in SLE T cells, and the distinct composition of lipid rafts has been shown to affect the outcome of T cell responses in SLE (4, 12). Third, the kinetics of two TCR-induced signaling pathways involving Syk kinases, namely actin polymerization and calcium signaling, are enhanced in SLE T cells (4), suggesting a possibility of differential involvement of these kinases in abnormally regulating these events. Therefore, we compared the expression and molecular associations of Syk and ZAP-70 between normal and SLE T cells. We observed that Syk was expressed in higher amounts in SLE compared with normal T cells and that SLE T cells displayed distinct binding of Syk and ZAP-70 to signaling molecules involved in actin polymerization and calcium signaling such as Vav, phospholipase C-γ1.

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‖ Abbreviations used in this paper: SLE, systemic lupus erythematosus; LAT, linker for activation of T cells; PLC-γ1, phospholipase C-γ1; PVDF, polyvinylidene difluoride; RA, rheumatoid arthritis; SS, Sjögren’s syndrome; SLEDAI, SLE disease activity index; Syk, spleen tyrosine kinase.

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Table I. Details of the patients involved in the study

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* Medications: ASA, aspirin (acetylsalicylic acid); CEL, celecoxib; HCQ, hydroxychloroquine; MTX, methotrexate; MYC, mycophenolate; PRED, prednisone.

(PLC-γ1), and linker for activation of T cells (LAT). These observations suggest that differential expression and association of Syk contributes to abnormal TCR signaling in SLE.

Materials and Methods
Patient samples and T cell isolation

Written informed consent was obtained from 58 SLE patients (51 females and seven males) with SLE disease activity indexes (SLEDAI) ranging from 0 to 16 and 52 healthy volunteers, as well as 9 rheumatoid arthritis (RA) and 9 Sjögren’s syndrome (SS) patients included in this study (Table I). The SLEDAI scores were calculated as originally described (13). The study protocol and the isolation of T cells using RosetteSep (StemCell Technologies) following the manufacturer’s instructions (4) have been described previously. With this technique we achieved a purity of >97% as assessed by CD3e staining and analysis by FACS. The study protocol was approved by the Health Use Committees of Walter Reed Army Institute of Research, Walter Reed Army Medical Center, University of Maryland, and Medstar Research Institute, and Beth Israel Deaconess Medical Center.

Western blotting, immunoprecipitation, and cytochalasin release experiments

The protocols for these experiments have been described previously (4, 10). Briefly, the cells were lysed with a buffer containing 1% Triton X-100. The supernatants were used for Western blotting and immunoprecipitation experiments. All samples were resolved on a 4–12% Bis-Tris NuPAGE gel (Invitrogen) and proteins were transferred onto polyvinylidene difluoride (PVDF) membranes and then incubated with specific Abs against phosphotyrosine (Upstate Biotechnology) or ZAP-70. Syk, PLC-γ1, β-actin, LAT (Santa Cruz Biotechnology), or p-Syk (clone I122-720; BD Biosciences). Another polyclonal anti-ZAP-70 Ab was a gift from Ronald Wange, National Institutes of Health, Bethesda, MD. P-coupled Abs (Santa Cruz Biotechnology) were used as secondary Abs, and detection was performed with ECL (Amersham Pharmacia Biotech). The detergent-insoluble, pelleted fractions left after cell lysis were used for eluting actin-bound proteins by the addition of 0.1 mg/ml cytochalasin B for 1 h at 37°C as described previously (4).

Actin polymerization assay

One million normal and SLE T cells were treated with either DMSO or 2 μM R406 for 1 h at 37°C. The cells were activated with anti-CD3 IgM Ab (a gift from Dr. D. Farber, University of Maryland School of Medicine, Baltimore, MD) for various time points and stained with phalloidin-FITC as described before (4). The peak polymerization for each time point was calculated as the ratio between the peak at each time point and the peak at 0 s. The shift in the peak between 30 s and 1 min was calculated as the ratio between peaks at 1 min and 30 s.

Intracellular Ca²⁺ concentration response analysis

These assays were performed as described previously (4); 5 × 10⁶ cells were treated with 2 μM R406 or equal volumes of DMSO for 1 h at 37°C and incubated with 1 μg/ml Indo-AM (acetoxyethyl ester) ( Molecular Probes) for 30 min at 37°C. Samples were run and at 30 s either OKT3 (10 μg/ml) or the isotype control murine IgG2a was added followed by goat anti-mouse cross-linker at 1 min or only PMA and 0.5 μg/ml ionomycin (Sigma-Aldrich), and the ratio of the fluorescence, which is directly proportional to free cytosolic Ca²⁺, was recorded for a period of 400 s as described previously using an Epics Altra (Beckman Coulter) flow cytometer (3, 14).

Densitometry and statistical analysis

Densitometric analysis of the autoradiograms was performed with the software program GelPro (Media Cybernetics). Statistical analysis of the data was done by Student r test using the software MINITAB, version 14 (Minitab). A value of p < 0.05 was considered to be significant.

Results

Increased expression and activity of Syk kinase in SLE T cells

It has been reported that in a majority of SLE patients, the peripherally circulating T cells demonstrate significantly diminished expression of the TCR ζ-chain, which instead is replaced by a homologous protein, FcRγ, that associates with Syk kinase (5). Because ZAP-70 has been shown to be the predominant kinase associated with TCR ζ signaling in normal T cells, we explored whether the expression of these two kinases was altered in SLE T cells compared with normal T cells. Western blot analysis of lysates derived from normal and SLE T cells demonstrated that while the expression of ZAP-70 remained virtually unaltered between normal and SLE T cells, the amount of Syk expression was increased by >23-fold (p < 0.05) in SLE T cells in >80% of cases (Fig. 1A and B). Simultaneously, however, we could not detect significant differences in the expression of Syk between normal and RA and SS T cells (Fig. 1C), suggesting that this observation was limited to SLE. The level of expression of Syk in SLE T cells was unaffected by the disease activity and we did not detect differences in the expression of Syk between cells derived from patients with a wide range of SLEDAI scores ranging from 0 to 16.

Next we asked whether, in addition to increased expression of Syk in SLE, the kinase activity of Syk was increased in SLE. Because phosphorylation of Syk confers activation of this kinase, we compared the expression of phosphorylated Syk in normal and SLE T cells activated with anti-CD3. As expected, we detected phosphorylation of Syk exclusively following activation of normal and SLE T cells with anti-CD3 Ab. In addition, phosphorylated Syk expression was significantly higher in SLE T cells stimulated with an IgM anti-CD3 Ab (Fig. 1D). We also performed intracellular staining experiments with anti-Syk Ab using FACS to determine the pattern of Syk staining within the T cell subsets in SLE. We noted a uniform pattern of staining of Syk within entire T cell...
Figure 1. Expression and activity of Syk are increased in SLE T cells. A, Western blot of 2 μg of proteins derived from normal and SLE T cells and resolved on a 4–12% Bis-Tris NuPAGE (Invitrogen), blotted to PVDF membrane, and probed with anti-Syk and β-actin Abs. B, Similar blot probed with anti-ZAP-70 Ab. The graphs in the bottom panel show the ratio of Syk:actin of densitometric values. *, p < 0.05 by Student's t test. C, Western blot of lysates derived from normal, RA, and SS cells, probed with Syk, ZAP-70, and β-actin Abs. D, Normal or SLE T cells were stimulated with an IgM anti-CD3 Ab for 15 and 30 s. Representative and cumulative data are shown. N, Normal; L, SLE; p-Syk, phosphorylated Syk.

Previously, we had shown that Syk but not ZAP-70, associated with lipid rafts in SLE T cells, suggesting that these kinases associate differentially with the actin cytoskeleton. To address this possibility, we compared the pattern of localization of Syk and ZAP-70 in SLE and normal T cells in the cytoplasmic and actin-bound fractions. We observed that in SLE T cells, high amounts of Syk could be detected within both cytoplasmic and actin-bound fractions, whereas in normal T cells very low amounts of Syk were detected within the cytoplasmic and actin fractions. Treatment with anti-CD3 Ab resulted in more significant mobilization of Syk to the actin-bound fractions in SLE compared with normal T cells (Fig. 2C). In sharp contrast, the amount of ZAP-70 that associated with the actin-bound protein fraction of SLE T cells was lower compared with that observed in normal T cells. Taken together, these observations suggest that in SLE T cells, Syk plays an important role in regulating signaling events closely associated with the actin cytoskeleton.

Role of Syk in TCR-induced signaling in SLE T cells

To test the possible role of Syk in the enhancement of TCR-induced signaling in SLE, we studied the effect of specific inhibition of Syk using the Syk-selective inhibitor R406 (15) on two cytoskeleton-regulated events, namely polymerization of β-actin and TCR-induced calcium response. We chose this compound over piceatannol because it is more selective for Syk kinase and therefore provided greater flexibility with dosing ranges compared with piceatannol, which exerts different effects on Syk and ZAP-70 at different doses. We had previously shown that the peak β-actin polymerization following treatment with anti-CD3 IgM Ab occurred at 30 s in SLE T cells, whereas it occurred at 1 min in normal T cells (4). In the present study we observed that when SLE T cells were treated with DMheatmap, the peak actin polymerization was noted at 30 s, consistent with
our previous observations (Fig. 3 and Table II). However, inhibition of Syk following treatment with R406 resulted in retardation of the kinetics of actin polymerization in SLE T cells, with the peak actin polymerization observed at 60 s. This difference in the shift of peaks between DMSO and R406 treatments was statistically significant (Fig. 3 and Table II; $p = 0.011$). However, the kinetics of actin polymerization in normal T cells remained minimally altered; there was in fact some statistically insignificant acceleration of the kinetics of actin polymerization, with peak actin polymerization occurring at 30 s in the presence of R406 ($p = 0.092$). T cells derived from patients with RA and SS showed kinetics of actin polymerization similar to that observed in normal T cells (Fig. 3 and data not shown). This retardation of the kinetics of β-actin polymerization exclusively in SLE T cells suggests that Syk plays a role in regulating actin-polymerization events in SLE but not normal T cells. In this experiment, we used T cells derived from SLE patients with SLEDAI scores ranging from 0 to 16 but observed consistent results, suggesting that these results are not affected by differences in the disease activity.

Similarly, culturing SLE and normal T cells in the presence of R406 resulted in profound suppression of intracellular calcium signaling in SLE (65% reduction) but not normal T cells (13% reduction), the difference being statistically significant ($p = 0.005$). This finding demonstrates the important role of Syk in the calcium signaling pathway exclusively in SLE (Fig. 4A). Although the magnitude of calcium responses were lower in RA T cells compared with normal T cells, the kinetics of calcium induction observed in RA T cells were similar to those observed in normal T cells (Fig. 4A). Although these results suggest Syk as the predominant kinase in calcium signaling in SLE, the lack of complete suppression of calcium signaling in the presence of R406 in SLE T cells suggested that possibly other kinases, including ZAP-70, have an overlapping role in mediating TCR-induced calcium responses in SLE T cells. These results were also consistently observed in T cells derived from patients with varying disease activity (SLEDAI ranging from 0 to 16). We observed that while R406 treatment suppressed TCR/CD3 complex-induced calcium responses in SLE, bypassing the TCR by PMA-ionomycin treatment resulted in high intracellular calcium responses, suggesting that the R406-induced suppression of calcium responses observed in SLE T cells was not due to the depletion of intracellular calcium by R406 (Fig. 4C).

**Differential associations of Syk and ZAP-70 in SLE T cells**

To understand the mechanism by which Syk contributes to heightened kinetics of cytoskeletal events, we compared the molecular associations of Syk and ZAP-70 in SLE and normal T cells. Specifically, we studied the association between Syk and three substrates of ZAP-70/Syk kinases, namely Vav, LAT, and PLC-γ1. Vav is an important protein that is required in F-actin polymerization and TCR capping. In addition, Vav is also required for optimal calcium mobilization via its role in the production of phosphatidylinositol 4,5-bisphosphate (PIP2), a substrate of PLC-γ1 (16, 17). LAT is critical to the calcium
responses via coupling of the TCR complex to the PLC-γ1/calcium signaling pathway (18). Immunoprecipitation studies demonstrated that the association between Syk and Vav was ~10-folds higher in SLE T cells compared with normal T cells (Fig. 5A; p = 0.006). Stripping and reprobing the blots with anti-phosphorylated Vav Ab revealed that Vav was inducibly phosphorylated in both normal and SLE T cells, with modestly more intense phosphorylation bands noted in SLE compared with normal T cells following anti-CD3 Ab treatment. The association between ZAP-70 and Vav was not found to be significantly different between normal and SLE T cells (Fig. 5A).

Next, we evaluated the association between Syk/ZAP-70 and LAT in normal and SLE T cells. Immunoprecipitation of LAT resulted in coprecipitation of similar amounts of Syk with LAT in normal and SLE T cells (Fig. 5B). However, while normal T cells demonstrated high amounts of LAT binding with ZAP-70, the amount of LAT that associated with ZAP-70 in SLE T cells was significantly reduced in a majority of SLE T cells (Fig. 5B).

**FIGURE 4.** Inhibition of Syk kinase by R406 dampens TCR-induced calcium response in SLE T cells. **A,** One million normal, SLE, and RA T cells were treated with either DMSO or 2 μM R406 for 1 h at 37°C and loaded with Indo-AM, and calcium flux in response to stimulus with anti-CD3 (OKT3) was analyzed for 400 s using Epics Altra (Beckman Coulter). **B,** The graph shows the percentage reduction of peak calcium response calculated as the difference in the heights of peak calcium response to DMSO and R406 treatment divided by the height of peak calcium response to DMSO treatment; n = 3 for normal and n = 4 for SLE and RA cells. **C,** Normal and SLE T cells treated with R406 as described above were further treated with PMA and ionomycin and the pattern of calcium flux was studied.

### Table II. F-actin polymerization in normal, SLE T and RA cells treated with R406

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<th>SLE R406 (MFI Ratio)**</th>
<th>RA DMSO (MFI Ratio)**</th>
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*Mean fluorescence ratio (MFI) = (MFI at the time point)/(MFI at 0 s).

**p = 0.092.

**p = 0.011.

***p = 0.134.
ALTERING THE OUTCOME OF SIGNALS EMANATING FROM THE TCR IN SLE CELLS RAISED THE POSSIBILITY THAT SYK PLAYS AN IMPORTANT ROLE IN NOT ZAP-70 ASSOCIATED WITH LIPID-RAFTS EXCLUSIVELY IN SLE T CELLS

Mechanisms that contribute to the pathological amplification of TCR-induced signaling remain unclear. We and others have reported that the structure of TCR is altered in a majority of SLE T cells (reviewed in Ref. 19). The finding that Syk associated with FcR through lipid rafts. Previously, we had shown that following ligation of the TCR/CD3 complex, SLE T cells displayed overall increases in intracellular tyrosine phosphorylation events (3). The observation in our present study that the inhibition of Syk kinases reduced the overall level of tyrosine phosphorylation in SLE T cells (Fig. 2A) suggests that enhanced activity of Syk kinases contributes to increased tyrosine phosphorylation of key signaling molecules that shape the outcome of TCR-induced signaling.

Previously, we had shown that following ligation of the TCR/CD3 complex, SLE T cells displayed overall increases in intracellular tyrosine phosphorylation events (3). The observation in our present study that the inhibition of Syk kinases reduced the overall level of tyrosine phosphorylation in SLE T cells (Fig. 2A) suggests that enhanced activity of Syk kinases contributes to increased tyrosine phosphorylation of key signaling elements in SLE.

The integrity of the actin cytoskeleton is essential for efficient formation and dissolution of immunological synapse. Also, the mobilization of lipid rafts and proteins bound to the actin cytoskeleton have been shown to play a major role in regulating signaling through lipid rafts. Thus, the increased association of Syk with actin compared with the association between ZAP-70 and actin (Fig. 2B) might explain in part why lipid rafts from SLE T cells exclusively contain Syk but not ZAP-70. The finding that selective inhibition of Syk resulted in reversal of the pathologically accelerated kinetics of F-actin polymerization and suppressed signaling events demonstrated by the cytoskeleton such as TCR-induced calcium signaling; and 4) Syk and ZAP-70 displayed differences in their patterns of association with key signaling molecules that shape the outcome of TCR-induced signaling.

Comparison of the expression of PLC-γ1 in normal and SLE T cells revealed that from similar amounts of protein lysates derived from normal and SLE T cells, similar amounts of PLC-γ1 could be immunoprecipitated with Abs against PLC-γ1 in SLE T cells (Fig. 5C). However, PLC-γ1 showed much higher binding to Syk (Fig. 5C) in SLE T cells compared with normal T cells that were activated with anti-CD3. Association between PLC-γ1 and β-actin remained unchanged between normal and SLE T cells. Similar to Syk, the association between PLC-γ1 and ZAP-70 was also enhanced in SLE compared with normal T cells (five of eight patients; Fig. 5, C and D). These results suggest that both Syk and ZAP-70 are involved in the regulation of TCR-induced calcium flux in SLE and are consistent with our earlier observation that the inhibition of Syk alone could not completely inhibit the calcium pathway in SLE T cells (Fig. 4A).

**Discussion**

Mechanisms that contribute to the pathological amplification of TCR-induced signaling remain unclear. We and others have reported that the structure of TCR is altered in a majority of SLE T cells, wherein TCR ξ is replaced by FcRγ (reviewed in Ref. 19). The finding that Syk associated with FcRγ and that Syk but not ZAP-70 associated with lipid rafts exclusively in SLE T cells raised the possibility that Syk plays an important role in altering the outcome of signals emanating from the TCR in SLE (4). In this study, we provide several lines of evidence that support this hypothesis as follows: 1) expression of Syk but not ZAP-70 is enhanced in SLE; 2) compared with ZAP-70, Syk demonstrated greater association with the actin cytoskeleton in SLE T cells; 3) inhibition of Syk “normalized” the kinetics of actin polymerization and suppressed signaling events demonstrated by the cytoskeleton such as TCR-induced calcium signaling; and 4) Syk and ZAP-70 displayed differences in their patterns of association with key signaling molecules that shape the outcome of TCR-induced signaling.

The samples were resolved on a 4–12% Bis-Tris gel and blotted to PVDF membranes and the blot was probed with Abs against individual proteins as shown on the side of each panel. IP, Immunoprecipitation; IB, immunoblot; IgH, Ig H chain; N, normal T cells; SLE, SLE T cells; p-Vav, phosphorylated Vav. To calculate p values, densitometric ratios were subjected to Student t test.

$ p = 0.012$). Again, we did not notice differences in the pattern of the binding of ZAP-70 with LAT between patients with different disease activity (SLEDAI ranging from 0 to 8). Because LAT is an important resident of lipid rafts, these findings are consistent with our previous observations that Syk alone but not ZAP-70 associated with lipid rafts in SLE T cells (4) and provide a plausible mechanism by which Syk augments signaling through lipid rafts.

**FIGURE 5.** Differential association of Syk with Vav (A), LAT (B), and PLC-γ1 (C and D) in SLE and normal T cells. One hundred micrograms of proteins derived from lysis of normal or SLE T cells were subjected to immunoprecipitation with Abs against signaling proteins as indicated in the figure. The samples were resolved on a 4–12% Bis-Tris gel and blotted to PVDF membranes and the blot was probed with Abs against individual proteins as shown on the side of each panel. IP, Immunoprecipitation; IB, immunoblot; IgH, Ig H chain; N, normal T cells; SLE, SLE T cells; p-Vav, phosphorylated Vav. To calculate p values, densitometric ratios were subjected to Student t test.
function, and Vav has been shown to play an important role in the kinetics of TCR/CD3 receptor capping (16, 17, 20). The observation that in normal T cells ZAP-70 is the predominant kinase that binds to Vav, but in SLE T cells both Syk and ZAP-70 bind in high amounts to Vav (Fig. 5A, top and bottom panels) with possible further enhancement of its activity, lends another rationale for the increased kinetics of the TCR-induced actin polymerization observed in SLE T cells.

TCR-induced calcium signaling is another intracytoplasmic event that is augmented in SLE. Our observation that suppression of Syk resulted in significant reduction in the calcium response suggests a critical role of Syk in the pathological augmentation of TCR-induced calcium flux in SLE (Fig. 4). Detection of association between Syk and PLC-γ1 exclusively in SLE T cells is significant in this regard (Fig. 5C). Similarly, LAT has been shown to couple the TCR to PLC-γ1 and thus participate in the calcium signaling (18). Compared with normal T cells, SLE T cells demonstrate decreased association between ZAP-70 and LAT, whereas Syk associates with LAT in high amounts (Fig. 5B), suggesting that Syk is the predominant kinase that regulates signaling events involving LAT such as calcium responses in SLE T cells.

It is not clear how precisely distal signaling leading up to IL-2 production is affected because of the differences in the utilization of Syk and ZAP-70 in the proximal signaling pathways in SLE. Differential regulation of the calcium-regulated pathways that are critical to IL-2 gene expression is a possible explanation (21, 22). Further studies are necessary to address this issue. Also, it remains unclear why Syk expression is increased in SLE T cells. Previously, we had drawn comparisons between SLE T cells and effector T cells that share many common features, including down-regulation of TCRζ and up-regulation of FcγR, demonstration of membrane clustering of TCR/CD3 complex at baseline, and even demonstration of the increased expression of Syk by effector cells and association of the active form of Syk to lipid rafts in SLE T cells (4). We had argued that conceivably the autoimmune process in SLE largely accounts for these observations by driving the T cells into a perpetual state of activation. Our present studies suggest that in this context, controlling signaling pathways involving Syk would be an important means of controlling the abnormal T cell activity in SLE.

More than a decade ago it was proposed that Syk and ZAP-70 might play different roles in different subsets of T cells (23). Subsequently, the flexibility of TCR-signaling in various immunological contexts has been observed by a simple switch in the utilization of Syk and ZAP-70 by the TCR. For example, in the thymus Syk is expressed more in early thymocytes and subsequently undergoes relative down-modulation in response to pre-TCR-induced signals, whereas ZAP-70 appears later during the transition of CD4+CD8− to single positive cells, a stage at which TCRζ signaling is established (23). In the periphery, the circulating naive and memory T cells in both humans and mice express high amounts of ZAP-70 and low amounts of Syk. As observed above, in vitro generated effector CD4 T cells preferentially used Syk instead of ZAP-70 (10). More recently, the double negative regulatory T cells that display TCRαβ+CD4−CD8− and are involved in suppressing host defenses against a transplanted organ were reported to signal through the FcγR—Syk pair (24). The observation that FcγR binds to Syk in SLE T cells suggests that the utilization of Syk by the TCR signaling machinery might be significant in certain autoimmune contexts as well. It is unclear why ZAP-70 is the predominant kinase that participates in TCR signaling in normal T cells despite the presence and activation of Syk kinase, albeit at lower levels. Our observation that Syk is excluded from the lipid rafts in normal T cells might be significant in this regard and might provide a mechanism by which the activity of Syk is restricted by normal T cells.

In summary, we show here that in SLE T cells there is higher expression of Syk kinase compared with normal T cells. This observation is also associated with alterations in the associations of Syk and ZAP-70 in SLE T cells, where there is a greater association of Syk with key signaling molecules that shape T cell responses. Although the precise outcome of these changes remains unknown, they might be involved in abnormal T cell responses such as the defective production of IL-2 or the sustained activation of T cells (10, 11). Therefore, we propose that Syk could serve as an important molecular target for controlling abnormal T cell activation in SLE.

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Disclosures
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