Syk Mutation in Jurkat E6-derived Clones Results in Lack of p72^{syk} Expression*

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J. Joseph Fargnoli, Anne L. Burkhardt, Maureen Laverty, Stephanie A. Kut, Nicolai S. C. van Oers, Arthur Weiss, and Joseph B. Bolen

From the 1Department of Oncology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543, the 2Howard Hughes Medical Institute, Department of Medicine, and Department of Microbiology and Immunology, University of California, San Francisco, California 94006, and the 1Department of Cellular Signaling, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California 94304

The human leukemic Jurkat cell line is commonly used as a model cellular system to study T lymphocyte signal transduction. Various clonal derivatives of Jurkat cells exist which display different characteristics with regard to responses to external stimuli. Among these, the E6-1 clone of Jurkat cells has been used as a parental line from which numerous important somatic mutant clones have been generated. During the course of experiments examining signals initiated by the T cell antigen receptor in an E6-1-derived Jurkat cell clone J.CaM1, we observed that the 72-kilodalton Syk protein tyrosine kinase previously found in other Jurkat cell lines was not detected. Upon further analysis it was determined that Syk transcripts from the J.CaM1 cells as well as the parental E6-1 cells contain a single guanine nucleotide insertion at position 92. This nucleotide insertion results in a shift in the Syk open reading frame leading to alternate codon usage as well as the generation of a termination codon at position 109. Thus, Syk transcripts in E6-1 cells and E6-1-derived clones are predicted to be incapable of encoding only the first 33 amino acids of the 630-amino acid wild type Syk. These findings are incompatible with a recently proposed model of T cell antigen receptor signal transduction based, in part, on experiments conducted using E6-1-derived cells, suggesting that Syk might play a role upstream of Lck and Zap70.

The Syk protein tyrosine kinase together with the Zap70 protein tyrosine kinase comprise a family of cytoplasmic enzymes that are important for signal transduction initiated by different types of surface receptors in cells of hemopoietic origin. Unlike other cytoplasmic protein tyrosine kinases, Syk and Zap70 possess tandem SH2 domains amino-terminal to their catalytic domain. The Syk and Zap70 SH2 domains serve to bind tandem phosphotyrosine containing elements in the membrane-associated signal coupling subunits of immune recognition receptors. These 18–20 amino acid elements are referred to as immunoreceptor tyrosine activation motifs and have the consensus sequence D/E�XXX(L/X)_{6,7}XXX(L/X) (4). Association of Syk and Zap70 with the phosphorylated immunoreceptor tyrosine activation motifs serves to position the kinases in a membrane proximal location and contribute to the activation of the enzymes (5–10).

Zap70 is expressed in all major thymocyte populations as well as in mature T cells of both CD4+ and CD8+ lineages (11). Syk too is expressed in all of the major thymocyte populations although the levels of Syk diminish severalfold in peripheral T lymphocytes (11). Syk is also expressed in an additional number of hemopoietic cells including B cells (11, 12), mast cells (13, 14), neutrophils (15), macrophages (16), erythroid cells (17), and platelets (18). Current evidence suggests that Zap70 and Syk may potentially contribute in distinct ways to signal transduction events in T cells and thymocytes. Patients with mutations in the Zap gene demonstrate abnormal development of thymocytes leading to the production of exclusively CD4+ T cells in the periphery (19–21). These T cells are, however, unresponsive to mitogen and antigen stimulation (19–21). While no SYK mutations have been documented in humans, targeted disruption of the syk gene in mice has been shown to block B cell but not T cell developmental pathways (22).

Much of our understanding of the signaling events initiated following the surface engagement of the T cell antigen receptor (TcR)^1 has been based upon model cell systems. One of the most useful and widely studied of these T cell models has been the human T cell leukemia line J urkat. Indeed, Zap70 was initially identified as a TcR Y subunit-associated protein and molecularly cloned from J urkat T cells (2, 23). Previous studies have demonstrated that J urkat T cells express both Syk and Zap70 (8, 11). Following TcR cross-linking both Syk and Zap70 were found to be enzymatically activated in a temporally indistinguishable manner (8) and both were found to be capable of association with the TcR Y subunit (8, 11). During the course of experiments examining protein tyrosine kinase signaling in the J.CaM1 somatic cell mutant isolated from the J urkat E6-1 line (24), we noticed that the 72-kDa Syk protein tyrosine kinase was not readily detected. On further analysis it was found that J.CaM1, the parental J urkat E6-1 line, as well as another E6-1-derived cell line J45.01 (25), express Syk transcripts containing a single guanine nucleotide insertion at position 92 which results in a frameshift leading to premature termination of the Syk open reading frame at position 109. In contrast, all Syk transcripts from other J urkat lines in which p72^{syk} was readily detected were found to encode wild type Syk. These results demonstrate that functional Syk is not expressed in all J urkat T cell-derived clones.

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¶To whom correspondence should be addressed: DNAX Research Institute of Molecular and Cellular Biology, 901 California Ave., Palo Alto, CA 94304. Tel.: 415-852-9196; Fax: 415-496-1200.

¶¶The abbreviations used are: TcR, T cell antigen receptor; PCR, polymerase chain reaction; SH2, src homology 2.
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MATERIALS AND METHODS

Cells—J. CaM1 is a clone derived from J urkat-FHCRC (26). J. CaM1 (for J urkat-derived Ca\(^{2+}\) mutant 1) is a derivative of E6-1 isolated following ethyl methanesulfonate treatment (24). J.45.01 is a CD45-deficient clone derived from the E6-1 clone following \(\gamma\) irradiation and anti-CD45 plus complement-mediated selection (25). H33HJ J A1 J urkat cells were purchased from ATCC. Other J urkat cells were obtained from Ivan D. Horak (National Cancer Institute), Gerald R. Crabtree (Stanford University), and Michel Nussenzweig (Rockefeller University). All J urkat cells were propagated in RPMI 1640 supplemented with 10% fetal bovine serum.

Antibodies—Rabbit antisera directed against Syk amino acids 260–370 and Zap70 amino acids 255–345 have been previously described (8). Antiseras against Syk amino acids 1–370 were produced by immunizing rabbits with the glutathione S-transferase-Syk fusion protein (Pharmacia Biotech Inc.).

Biochemical Analyses—Cell lysis, Syk and Zap70 immunoprecipitation, immune-complex kinase assays, and immunoblotting have all been previously described (8).

RNA Isolation and Northern Analysis—Total RNA was isolated on an ABI 3100 GENEPURE automated extractor (Perkin Elmer Corp.) and used directly for PCR or enriched for polyadenylated RNA by two cycles of oligo(dT) chromatography on prepoured columns according to the manufacturer’s recommendations (Life Technologies). Radiolabeled cDNA probes corresponding to the entire open reading frame of human Syk or chicken \(\beta\)-actin (27) were generated using \(\gamma\)-\[^32\]P\]dCTP (3000 Ci/mmol, DuPont NEN) and the RadPrime Labeling System (LifeTechnologies). Ten micrometres of enriched polyadenylated RNA was used for Northern analysis by fractionation on 1.1% agarose gels and transferred to Hybond N \(^\oplus\) nitrocellulose (Amerham) using 10 \(\times\) SSC (1.5 \(\mu\) sodium chloride, 0.15 \(\mu\) sodium citrate). Hybridization and wash conditions were carried out under high stringency conditions according to Sambrook et al. (28).

PCR Cloning and Sequencing—Five micrograms of total RNA was reverse transcribed with random hexamers (1 \(\mu\)l of 100 ng/\(\mu\)l/reaction) using Superscript II reverse transcriptase according to the manufacturer’s recommendations (LifeTechnologies). Five microliters of the reverse transcription reactions were used for PCR with Hot Tub \(^\oplus\) polymerase (Amerham) using primers with homology to various positions in the Syk cDNA as indicated in the text. For PCR of the internal DNA fragments surrounding the mutation site, 35 cycles of a 20-s denaturation step at 94 °C, an annealing step at 55 °C for 20 s, and an extension for 40 s at 70 °C was used. For PCR of the entire open reading frame, two-step PCR (29) was employed using 35 cycles with an 4-s denaturation step at 94 °C, an annealing step at 55 °C for 20 s, and an extension for 40 s at 70 °C. Primer pairs were designed to amplify Syk from H33HJ (left panel) or Zap70 (right panel) for the H33HJ cells. Primer sequences are given below the nucleotide sequences with the H33HJ amino acid sequences above those predicted for the E6-1 amino acid sequences. The numbers on the left refer to nucleotide positions within the open reading frame. The Syk sequence derived from the H33HJ cells is the same as that previously determined from cDNAs isolated from Daudi Burkitt’s lymphoma cells (30).

RESULTS

Lack of Detectable \(p72^{\text{Syk}}\) in E6-1-derived J urkat Cell Lines—Representative J urkat cell lines were examined for the expression of Zap70 and Syk by specific enzyme immunoprecipitation followed by immunoblot analysis. As shown in Fig. 1 (left panel), all of the J urkat lines were found to express approximately equivalent amounts of Zap70. The results shown in Fig. 1 (right panel) demonstrate that \(p72^{\text{Syk}}\) expression was not detected in the E6-1 J urkat clone nor in the E6-1-derived J. CaM1 clone while Syk was easily detected in the H33HJ J urkat clone as well as all other non-E6-1-derived J urkat cells we have tested (data not shown). We also failed to detect Syk expression in the E6-1-derived J.45.01 clone (data not shown). In other experiments, we found that Syk was not detected by immunoblotting in the E6-1, J. CaM1, or J.45.01 cells using other Syk antisera directed to different portions of the enzyme nor did we detect Syk by measuring autophosphorylating activity in immune complex protein kinases assays (data not shown). Furthermore, we failed to detect Syk activity or protein expression following TcR cross-linking in the E6-1-derived cell lines (data not shown). These results indicate that the Syk protein is not expressed prior to or following TcR mediated activation in the E6-1-derived cell lines.

Detection of Syk Transcripts in E6-1-derived J urkat Cell Lines—Poly(A)\(^\oplus\) RNA was isolated from the various J urkat cell lines and Northern analysis was performed to determine if Syk mRNA’s were expressed. The results of this experiment (Fig. 2, 3).
numbers refer to positions of primers relative to the initiation codon 

mutated Syk open reading frame allows for the predicted trans-

termination of the open reading frame at position 109. The alter-

native usage of two codons before directing the premature 

Syk open reading frame. The resulting frameshift allows for 

detectable Syk in these cells is at least in part the conse-

sequence of the preceding experiments raised the possibility that the 

open reading frame of Syk expressed in E6-1-derived cells 

might contain some type of mutation that prohibited stable 

protein production. To explore this possibility Syk cDNAs were 

isolated, cloned, and sequenced from representative E6-1 and 

non-E6-1 Jurkat backgrounds. As shown in Fig. 3 and docu-

mented further in Table I, all of the full-length Syk cDNA 
dones isolated from E6-1 lineage Jurkats were found to contain 
a single guanine nucleotide insertion at position 92, while the 
Syk cDNA sequences from non-E6-1 Jurkats were found to be 
identical to the wild type Syk (30). Additional partial cDNAs 
which included the portion containing the single nucleotide 
insertion were also independently isolated and sequenced. The 
results of these studies (Table I) confirmed that E6-1-derived 
Jurkats contain Syk transcripts with an additional guanine at 
position 92.

The consequence of the guanine insertion at position 92 predicts that the Syk open reading frame should be shifted and undergo premature termination at position 109 allowing for the potential production of an Syk peptide of only 35 amino acids. This severely truncated gene product would not be detectable with our Syk antisera. In keeping with this prediction, the results shown in Fig. 4 demonstrate that transcription/translation of the Syk open reading frame from the E6-1-derived cells failed to produce a detectable Syk protein while the Syk open reading frame obtained from non-E6-1-derived Jurkats produced the expected 72-kDa Syk protein.

**DISCUSSION**

The results presented in this report demonstrate that Jurkat cell clones derived from the J urkat E6-1 clone fail to detectably express a functional Syk protein tyrosine kinase. The absence of detectable Syk in these cells is at least in part the consequence of a guanine nucleotide insertion at position 92 in the Syk open reading frame. The resulting frameshift allows for alternative usage of two codons before directing the premature termination of the open reading frame at position 109. The mutated Syk open reading frame allows for the predicted tran-

Table I

| Sequence results of syk PCR cloning | No. clones | CDNA Rx
ttRNA | 6 × G (WT) | 7 × G |
|-----------------------------------|----------|-----------|----------|--------|
| J urkat. | 20 | 2 | + |
| J urkat.11/38 | 6 | 1 | + |
| J urkat.20/465 | 6 | 1 | + |
| J-cam | 8 | 2 | + |
| J-cam.11/38 | 6 | 2 | + |
| J-cam.20/465 | 6 | 1 | + |
| E-6 | 4 | 1 | + |
| E-6.11/38 | 6 | 1 | + |
| E-6.20/465 | 3 | 1 | + |

*a* CDNA reactions refer to independent pools of RNA used in reverse transcription reactions. 

*b* Expected wild-type sequence for syk at nucleotide position 92 of the open reading frame. 

*c* Sequence found in mutant syk at nucleotide position 92 of the open reading frame resulting in a termination codon at position 109. 

*d* fl is the entire coding plus non-coding sequence using primers beginning at −41 and +1392 relative to the initiation codon (+1) and numbers refer to positions of primers relative to the initiation codon used in the PCR reactions.

The lack of Syk expression in the E6-1 Jurkat cells does not appear to significantly alter the TCR-mediated responses of these cells when compared with J urkat clones expressing Syk. Moreover, these results, together with those obtained with mice deficient in Syk expression (22) as well as other studies analyzing E6-1 Jurkat signaling (2, 3, 7, 11, 23–25), argue against a previously proposed model placing Syk upstream of Lck and Zap70 (31, 32).

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