these mutations have pleiotropic effects. In addition, we find that the mutant receptor that lacks both phosphorylation sites, but binds 20% of the GAP bound to wild-type PDGFRs, does not stimulate DNA synthesis in response to PDGF, suggesting that association of a small quantity of GAP is insufficient to trigger a biological response.

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17. Dog kidney epithelial TRMP cells [M. S. Turker *et al.*, *Cell Biol. Toxicol.* **4**, 211 (1988)] that lack endogenous PDGFR were infected with a retroviral expression vector containing a human β subunit PDGFR cDNA and a neomycin phosphotransferase gene, and resistant populations were selected in G^{418} (*16*). The virus stocks used in this study were high titer stocks from cloned packaging cell lines, and the resulting epithelial cell populations expressed approximately ten times as many receptors per cell relative to those described previously (*16*). Immunoprecipitation of [^{35}S]methionine-labeled cells with PR7212 anti-PDGFR monoclonal antibody showed that TRMP cells expressing wild-type and mutant receptors had equivalent numbers of receptors to within a 10% range (*22*). TRMP cells infected with an empty vector showed no mitogenic response to PDGF, whereas those expressing the wild-type PDGFR were responsive, showing up to 40% of the response elicited by 10% serum (*22*).
18. PDGF was the BB isoform, purified to homogeneity from yeast cell cultures expressing a recombinant DNA clone.
19. Anti-GAP antibody was raised to residues 171–448 of GAP (*12*). Anti-PDGFR monoclonal antibody PR7212 recognizes an extracellular epitope [C. E. Hart, R. A. Seifert, R. Ross, D. F. Bowen-Pope, *J. Biol. Chem.* **262**, 10780 (1987)]. Anti-PY antibody 1G2 was used to immunoaffinity purify PY-containing proteins as described [R. D. Huhn *et al.*, *J. Cell. Biochem.* **39**, 129 (1989)].
20. SDS polyacrylamide gels contained 7.5% acrylamide and 0.19% bis acrylamide. For two-dimensional electrophoresis, immunoprecipitates were first subjected to isoelectric focusing at *p*H 3.5 to 10, then resolved in the second dimension on a 7.5% SDS polyacrylamide gel, as described (*21*). Immunoblots on Immobilon transfer membrane (Millipore) were probed with anti-GAP antiserum (*19*) and developed with an avidin, biotin, alkaline phosphatase detection system (Vector Laboratories).
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23. Cells were lysed in EB (*21*) and immunoprecipitated as previously described (*16*). Immunoprecipitates were phosphorylated in vitro, as described (*16*).
24. Phosphoproteins were extracted, digested with trypsin, and the phosphopeptides were resolved in two dimensions (*p*H 8.9 electrophoresis and chromatography in buffer 1), as described (*16*).

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A New Member of the Leucine Zipper Class of Proteins That Binds to the HLA DRα Promoter

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Several mutants derived from transformed human B cell lines are defective in expressing major histocompatibility complex (MHC) class II genes. The failure to express a class II gene in at least one such mutant line has been mapped to the MHC class II X box, a conserved transcriptional element in the promoter region. A complementary DNA encoding a DNA-binding protein (human X box binding protein, hXBP-1) whose target is the human DRα X box and the 3′ flanking region has now been cloned. This complementary DNA encoded a protein with structural similarities to the c-*jun* proto-oncogene product, and its target sequence was closely related to the palindromic target sequence of c-*jun*. Mutation of the hXBP-1 DNA target sequence decreased DRα promoter activity in vivo. These studies suggest that the hXBP-1 protein acts as a transcription factor in B cells.

TRANSCRIPTIONAL CONTROL OF EUkaryotic gene expression is usually mediated by sequence-specific transcription factors that bind to DNA. A number of DNA-binding activities specific for sequences upstream of class II genes have been identified; several of these factors bind to the highly conserved X box and Y box motifs of human (*1, 2*) and murine (*3–5*) class II genes. Experiments with mice bearing Eα transgenes have shown a functional role of X and Y box sequences in class II transcription; deletion of X or Y resulted in greatly diminished Eα expression as well as abnormal transcriptional start sites (*3, 6*). Furthermore, transfected class II genes containing the X box motif are only minimally transcribed in several human class II negative mutant B cells (*7–12*). For two such mutant lines, the defects in transcription mapped to the X box, suggesting a defect in the activity of proteins that act through this motif (*10, 12*). To isolate the genes and proteins that influence transcription through binding to the X box motif, we used a method developed by Singh *et al.* (*13*). A λgt11 human B cell cDNA expression library was screened with a high-affinity labeled X box target sequence. The oligonucleotide we used as a probe was X(Aα)44, a 44-bp oligonucleotide containing the murine AαX box as well as the interspace sequence separating the X and Y boxes. We had shown earlier that nuclear extracts from human B lymphoblastoid cell lines contained binding proteins that recognize residues within this 44-bp motif (*4*). From 750,000 plaques examined, we identified a clone, λhXBP-1, that remained positive after three rounds of screening and was negative with a control probe from an upstream region of the Aα gene.

To characterize the binding site and structure of λhXBP-1, we tested lysates made from λhXBP-1 for binding activity by gel retardation analysis; various oligonucleotides corresponding to human class II se-

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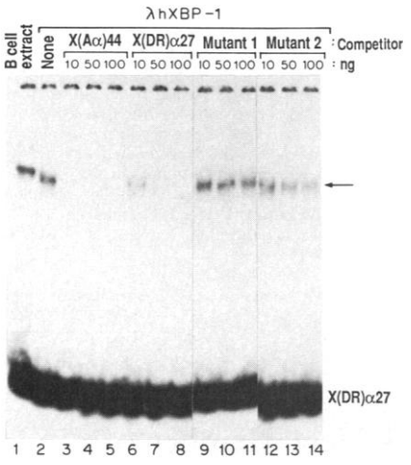

Fig. 1. λhXBP-1 recognizes the DRα promoter region. Gel retardation assays with λhXBP-1 lysates or with native extract from the human Raji B cell line were performed with labeled X(DRα)27 oligonucleotide as described (*18*). The reaction mixtures were resolved by electrophoresis on a 4% polyacrylamide gel in tris-borate EDTA buffer (TBE). The amount of competitor DNA was 10, 50, or 200 ng of X(Aα)44, X(DRα)27, mutant oligo 1, and mutant oligo 2. Oligonucleotides were prepared on Du Pont or BioSearch synthesizers by standard phosphoramidite techniques. After being dried under vacuum, the resulting DNA was end-labeled with [γ-^{32}P]adenosine triphosphate and polynucleotide kinase. Strands were annealed by boiling for 5 min in 0.5*M* NaCl, 20 m*M* tris-HCl, *p*H 7.5, and 1 m*M* EDTA and incubating overnight at 65°C. After ethanol precipitation, the annealed complex was isolated from an 8% gel in 1× TBE. The sequence of X(DRα)27 is CCTAGCAACAGATGCGTCATCTCAAAA. The sequence of mutant 1 is CCTAGCAACAGATGTACGTAGCAGCTG and mutant 2 is TACGTAGCAGCTGTCGTCATCTCAAAA. Cloning of λhXBP-1 from an expression library. λhXBP-1 phage library screening was done by the method of Singh *et al.* (*13*) and Huynh *et al.* (*19*) with modifications as previously described (*18*). A total of 7.5 × 10^5 plaque-forming units of the λgt11 library [human B cell cDNA library (Clontech)] were screened with the murine X(Aα)44 probe (*4*). The clones that bound X(Aα)44 but did not bind unrelated probes were designated λhXBP clones. Lysogens of λgt11 in *Escherichia coli* strain Y1089r$^-$ were formed, isolated, and then induced to overproduce their β-galactosidase fusion proteins with isopropyl β-D-thiogalactopyranoside and lysed to make lysogen extracts as described (*13, 19*).

quences were used because the X box motif and interspace elements of the members of the human class II family display variable degrees of sequence homology. Three of these sequences (DQ_α, DQ_β, and DR_β) did not bind the λhXBP-1 fusion protein (*14*). When an oligonucleotide containing the DRα X box and X-Y interspace element was used as probe, however, binding activity was present. The labeled DRα oligonucleotide probe [X(DRα)27] bound the hXBP-1 protein (Fig. 1, lane 2). The formation of the λhXBP-1–DRα complex was inhibited by the addition of unlabeled X(DRα)27 oligonucleotide (lanes 6 to 8) as well as unlabeled X(Aα)44 oligonucleotide (lanes 3 to 5). To determine whether the X box motif and interspace element of DRα were both involved in the complex formation, we used two additional oligonucleotides in gel retardation assays. These two oligonucleotides contained the DRα X box sequence, plus a scrambled, random sequence in place of the interspace element (mutant oligo 1) or a scrambled random sequence in place of the X box motif, plus the DRα interspace element (mutant oligo 2). Neither of these unlabeled oligonucleotides efficiently prevented the formation of the λhXBP-1–DRα complex (Fig. 1, lanes 9 to 14). We conclude from these results that sequences within the X box motif as well as sequences within the interspace element contribute to the formation of the hXBP-1–DRα complex.

Methylation interference experiments performed to better define the actual contact sites of λhXBP-1 on the DRα sequence showed that hXBP-1, like the proteins in native nuclear extract, contacted G residues both in the consensus X box element and in the X-Y interspace element (*14*). To show more directly which G residues are involved, we prepared two mutant oligonucleotides for competition experiments. Mutant oligonucleotide DR (4G*) changed some G residues to C or A residues (see legend to Fig. 2). Mutant oligonucleotide DR (2G*) changed the most 3′ G residue in the X box proper and the G residue in the interspace motif to A residues. Neither of these mutants effectively competed with the binding of proteins in native extract or in hXBP-1 lysate to DRα at 10 or 50 ng (and only slightly at 200 ng) in contrast to the wild-type oligonucleotide, which competed effectively at 50 ng (Fig. 2). These data show that the proteins in native nuclear extracts and hXBP-1 both bind to DRα DNA with a marked specificity for dG residues at the starred positions, as compared to methyl-dG or dA residues. We conclude that sequences within the X box motif as well as sequences within the interspace element contribute to hXBP-1–DRα complex formation. In other studies, however, Reith and his colleagues showed that the DRα X box binding protein, RF-X, which is missing in a subset of patients with severe combined immunodeficiency disease (SCID), contacts residues only in the X box motif itself, although the formation of the complex does require the interspace element as well as sequence upstream of the X box (*15*).

We performed transient transfection experiments to correlate in vitro binding assays with in vivo function. Mutation of the two most 3′ G residues in the X box proper and the most 5′ G residue in the interspace region resulted in a 50% decrease in DRα promoter activity relative to wild-type X box (Table 1). These data are similar to those of previous studies (*11*) in which mutation of the X consensus sequence at every base decreased DRα promoter activity by 30%. Mutation of these three bases, which are important for hXBP-1–DRα complex formation (Fig. 2), decreased promoter activity at least as much as mutating the complete X box proper.

The amino acid sequence of 260 residues deduced from the nucleotide sequence of the full-length hXBP-1 cDNA insert has several features characteristic of DNA-binding transcription factors of the leucine zipper class of proteins (Myc, Fos, Jun, cEBP, GCN4, and CRE-BP) (*16*). This newly defined structure has been shown to be responsible for dimerization between the *jun* and *fos* proto-oncogenes, and this dimerization is necessary for the binding to the AP-1 target sequence (*16*) and for transformation (*16*) (Fig. 3, A and B). Between positions 300 and 410 there is a hypothetical leucine zipper sequence in which six leucines are spaced seven residues apart. Adjacent to the heptad repeat of leucines is a basic domain in which 45% of the residues are either

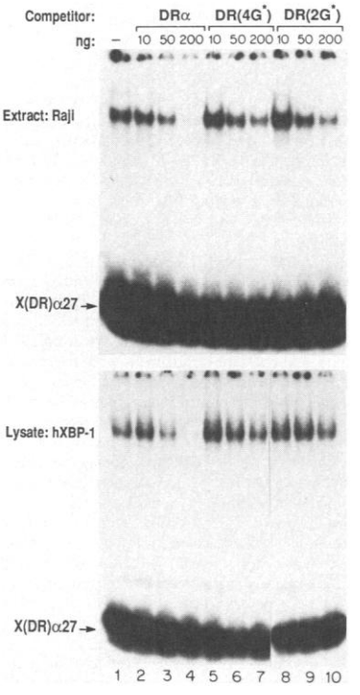

Fig. 2. Competition assays with mutated DRα oligonucleotides DR(4G*):CCTACCAACACATACATCATCTCAAAA and DR(2G*):CCTAGCAACAGATACATCATCTCAAAA. Underlined residues denote G replacement. The amount of competitor DNA used in the gel retardation assay was 10, 50, or 200 ng.

A

```
TAG TCT GGA GCT ATG GTG GTG GTG GCA GCC GCG CCG AAC CCG GCC GAC GGG ACC CCT AAA   60
                met val val val ala ala ala pro asn pro ala asp gly thr pro lys

GTT CTG CTT CTG TCG GGG CAG CCC GCC TCC GCC GCC GGA GCC CCG GCG GCC AGG CTG CCG  120
val leu leu leu ser gly gln pro ala ser ala ala gly ala pro ala ala arg leu pro

CTC ATG GTG CCA GCC CAG AGA GGG GCC AGC CCG GAG GCA GCG AGC GGG GGG CTG CCC CAG  180
leu met val pro ala gln arg gly ala ser pro glu ala ala ser gly gly leu pro gln

GCG CGC AAG CGA CAG CGC CTC ACG CAC CTG AGC CCC GAG GAG AAG GCG CTG AGG AGG AAA  240
ala arg lys arg gln arg leu thr his leu ser pro glu glu lys ala leu arg arg lys

CTG AAA AAC AGA GTA GCA GCT CAG ACT GCC AGA GAT CGA AAG AAG GCT CGA ATG AGT GAG  300
leu lys asn arg val ala ala gln thr ala arg asp arg lys lys ala arg met ser glu

CTG GAA CAG CAA GTG GTA GAT TTA GAA GAA GAG AAC CAA AAA CTT TTG CTA GAA AAT CAG  360
leu glu gln gln val val asp leu glu glu glu asn gln lys leu leu leu glu asn gln

CTT TTA CGA GAG AAA ACT CAT GGC CTT GTA GTT GAG AAC CAG GAG TTA AGA CAG CGC TTG  420
leu leu arg glu lys thr his gly leu val val glu asn gln glu leu arg gln arg leu

GGG ATG GAT GCC CTG GTT GCT GAA GAG GAG GCG GAA GCC AAG GGG AAT GAA GTG AGG CCA  480
gly met asp ala leu val ala glu glu glu ala glu ala lys gly asn glu val arg pro

GTG GCC GGG TCT GCT GAG TCC GCA GCA CTC AGA CTA CGT GCA CCT CTG CAG CAG GTG CAG  540
val ala gly ser ala glu ser ala ala leu arg leu arg ala pro leu gln gln val gln

GCC CAG TTG TCA CCC CTC CAG AAC ATC TCC CCA TGG ATT CTG GCG GTA TTG ACT CTT CAG  600
ala gln leu ser pro leu gln asn ile ser pro trp ile leu ala val leu thr leu gln

ATT CAG AGT CTG ATA TCC TGT TGG GCA TTC TGG ACA ACT TGG ACC CAG TCA TGT TCT TCA  660
ile gln ser leu ile ser cys trp ala phe trp thr thr trp thr gln ser cys ser ser

AAT GCC CTT CCC CAG AGC CTG CCA GCC TGG AGG AGC TCC CAG AGG TCT ACC CAG AAG GAC  720
asn ala leu pro gln ser leu pro ala trp arg ser ser gln arg ser thr gln lys asp

CCA GTT CCT TAC CAG CCT CCC TTT CTC TGT CAG TGG GGA CGT CAT CAG CCA AGC TGG AAG  780
pro val pro tyr gln pro pro phe leu cys gln trp gly arg his gln pro ser trp lys

CCA TTA ATG AAC TAA TTC GTT TTG ACC ACA TAT ATA CCA AGC CCC TAG TCT TAG AGA TAC  840
pro leu met asn ●

CCT CTG AGA CAG AGA GCC AAG CTA ATG TGG TAG TGA AAA TCG AGG AAG CAC CTC TCA GCC  900
CCT CAG AGA ATG ATC ACC CTG AAT TCA TTG TCT CAG TGA AGG AAG AAC CTG TAG AAG ATG  960
ACC TCG TTC CGG AGC TGG GTA TCT CAA ATC TGC TTT CAT CCA GCC ACT GCC CAA AGC CAT 1020
CTT CCT GCC TAC TGG ATG CTA CAG TGA CTG TGG ATA CGG GGG TTC CCT TTC CCC ATT CAG 1080
TGA CAT GTC CTC TCT GCT TGG TGT AAA CAT TCT TGG GAG GAC ACT TTT GCC AAT GAA CTC 1140
TTT CCC CAG CTG ATT AGT GTC TAA GGA ATG ATC CAA TAC TGT TGC CCT TTT CCT TGA CTA 1200
TTA CAC TGC CTG GAG GAT AGC AGA GAA GCC TGT CTG TAC TTC ATT CAA AAA GCC AAA ATA 1260
GAG AGT ATA CAG TCC TAG AGA ATC CCT CTA TTT GTT CAG ATC TCA TAG ATG ACC CCC AGG 1320
TAT TGC CTT TTG ACA TCC AGC AGT CCA AGG TAT TGA GAC ATA TTA CTG GAA GTA AGA AAT 1380
ATT ACT ATA ATT GAG AAC TAC AGC TTT TAA GAT TGT ACT TTT AAG ATT GTA CTT TTA TCT 1440
TAA AAG GGT GGT AGT TTT CCC TAA AAT ACT TAT TAT GTA AGG GTC ATT AGA CAA ATG TCT 1500
TGA AGT AGA CAT GGA ATT TAT GAA TGG TCT TTA TCA TTT CTC TTC CCC CTT TTT GGC ATC 1560
CTG GCT TGC CTC CAG TTT TAG GTC CTT TAG TTT GCT TCT GCA AGC AAC GGG AAC ACC TGC 1620
TGA GGG GGC TCT TTC CCT CAT GTA TAC TTC AAG TAA GAT CAA GAA TCT TTT GTG AAA TTA 1680
TAG AAA TTT ACT ATG TAA ATG CTT GAT GGA ATT TTT TCC TGC TAG TGT AGC TTC TGA AAG 1740
GTG CTT TCT CCA TTT ATT TAA AAA CTA CCC ATG CAA TTA AAA GGT ACA ATG CAA AAA AAA 1800
AAA AAA AAA AAT TTT TTT                                                        1818
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B

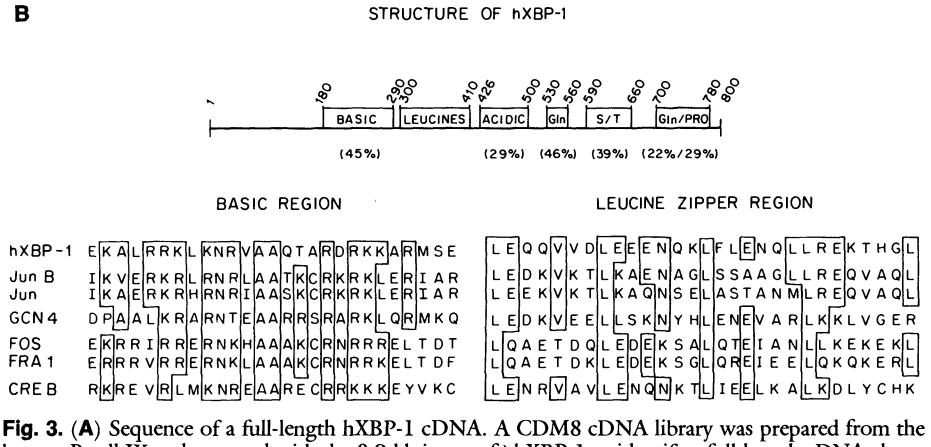

Fig. 3. (**A**) Sequence of a full-length hXBP-1 cDNA. A CDM8 cDNA library was prepared from the human B cell JY and screened with the 0.9-kb insert of λhXBP-1 to identify a full-length cDNA clone. This clone, JY 113, contained a 1.8-kb insert. The leucine residues and basic amino acids are circled or underlined. The acidic stretch (boxed) and the serine/threonine-rich region (dashed underline) are indicated. Dideoxy double-stranded sequence analysis was as described in the USB manufacturing manual. (**B**) Schematic diagram of hXBP-1 structure and similarity of hXBP-1 basic region and leucine repeat region to other members of the leucine zipper class of proteins. Indicated are percentages of residues that are in the various (numbered) regions.

Table 1. Transfection of human B cells with a DRα X box substitution mutant. Raji cells were transfected by electroporation with recombinant plasmids containing DRα promoter fragments fused to the bacterial chloramphenicol acetyl transferase (CAT) gene as described (*8*). The 5′ Δ-56X+Y plasmid contains the wild-type DRαX, Y, octamer, and TATA elements. DR (3G*) is the same as 5′ Δ-56X+Y, except the two most 3′ G residues of the X box and the 5′ G in the interspace region were changed to C or A residues (CCTAGCAACACATACATCATCTCAAAA). In 5′ Δ-56, both X and Y elements are deleted and pD164-2 contains no DRα promoter sequence (*11*). Extracts of transfected cells were analyzed for CAT activity by thin-layer chromatography and autoradiography. Conversion of chloramphenicol to its acetylated forms was quantified by scintillation counting of the radioactive spots. The data are normalized to CAT activity measured for 5′ Δ-56X+Y and are the average of five independent experiments ± SD.

Plasmid	Relative CAT activity
5′ Δ-56X+Y	1.00 (39.9%)*
DR (3G*)	0.45 ± 0.05
5′ Δ-56	0.22 ± 0.06
pD164-2	0.04 ± 0.03

*Actual average percent conversion of chloramphenicol to its acetylated forms.

arginine or lysine. The basic domain and leucine repeat region are similar to sequences from all members of the leucine zipper class of proteins. Specifically, there is 41% similarity in the basic region (Fig. 3B) and 34% in the leucine zipper region (Fig. 3B) with proteins encoded by c-*jun* and *jun* B (*17*). If conservative amino acid changes are discounted, this similarity increases to 74% in the basic region and 69% in the leucine zipper region. Six of seven bases in the target sequence of hXBP-1 (5′-TGCGTCA-3′) are identical to those of the palindromic target sequence of the c-Jun protein and GCN4 (5′-TGA$^{C}_{G}$TCA-3′). In addition, the hXBP-1 protein contains other motifs, such as an acidic stretch, a serine- and threonine-rich region, and glutamine-rich regions that are common to several transcription factors. The overall structure of the hXBP-1 protein as well as the in vivo transcription experiments (Table 1) suggest that the hXBP-1 protein acts as a transcription factor in B cells. This conclusion is strengthened by the observation that hXBP-1 mRNA levels are very low in a histocompatibility locus antigen (HLA)–DR^{-} mutant B cell line, 6.1.6 (*14*). Experiments to introduce antisense hXBP-1 sequences into normal B cells, as well as the hXBP-1 cDNA into the 6.1.6 mutant B cell line, should clarify the role of this DNA-binding protein in MHC class II gene regulation.

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T Cell Antigen Receptor–Mediated Activation of Phospholipase C Requires Tyrosine Phosphorylation

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Triggering of the antigen-specific T cell receptor–CD3 complex (TCR-CD3) stimulates a rapid phospholipase C–mediated hydrolysis of inositol phospholipids, resulting in the production of second messengers and in T cell activation and proliferation. The role of tyrosine phosphorylation in these events was investigated with a tyrosine protein kinase (TPK) inhibitor, genistein. At doses that inhibited TPK activity and tyrosine phosphorylation of the TCR ζ subunit, but not phospholipase C activity, genistein prevented TCR-CD3–mediated phospholipase C activation, interleukin-2 receptor expression, and T cell proliferation. These findings indicate that tyrosine phosphorylation is an early and critical event that most likely precedes, and is a prerequisite for, inositol phospholipid breakdown during receptor-mediated T cell activation.

ACTIVATION OF T LYMPHOCYTES BY ligands of the TCR-CD3 complex is associated with phosphoinositide-specific phospholipase C (PI-PLC)–mediated breakdown of inositol phospholipids; this gives rise to two second messengers, namely, inositol phosphates and diacylglycerol (*1*). The resulting increase in intracellular Ca^{2+} concentration and translocation of protein kinase C (PKC) to the plasma membrane are thought to initiate the cascade of biochemical events leading to activation of T cell effector functions and clonal expansion (*2, 3*). Triggering of the TCR-CD3 complex also induces tyrosine phosphorylation of a number of proteins (*4*), including the ζ subunit of this complex (*5*). This implies a role for a TPK in T cell activation. Although this tyrosine phosphorylation appears to be independent of inositol phospholipid hydrolysis (*6, 7*) and activation of PKC (*8*), the exact relationship between these two pathways and their relative contribution to the overall process of T cell activation remain unknown. In particular, it is not known whether the stimulation of PI-PLC and the subsequent production of inositol phospholipid–derived second messengers require a tyrosine phosphorylation event.

We have studied the role of tyrosine phosphorylation in signal transduction through the TCR-CD3 complex with the use of a TPK inhibitor, genistein. This isoflavone compound was found to specifically inhibit the epidermal growth factor receptor, $pp60^{c\text{-}src}$, and $pp110^{gag\text{-}fes}$ TPKs, whereas it had marginal effects on several serine and threonine kinases (*9*). To determine whether genistein inhibits TPKs in T lymphocytes, we tested it in three different assays. (i) We measured total TPK activity of T lymphocyte membranes that had been incubated for 10 min with various concentrations of genistein. The drug inhibited the kinase activity in a dose-dependent manner, with 50% inhibition being achieved at 30 μg/ml (Fig. 1A). (ii) The effect of genistein on the enzymatic activity of $pp56^{lck}$, a T cell–specific TPK (*10*), was evaluated by measuring the ability of $pp56^{lck}$ to phosphorylate a synthetic peptide substrate (Fig. 1B) or to become autophosphorylated (Fig. 1C). Autophosphorylation correlates with activity toward exogenous substrates (*10*). Both phosphorylation reactions were similarly inhibited in a dose-dependent manner with an inhibition constant (K_i) of 10 μg/ml. (iii) The effects of genistein on tyrosine phosphorylation in intact cells was assessed by measuring phytohemagglutinin (PHA)-induced TCR ζ chain phosphorylation (Fig. 2). T cell mitogens, antigen, and antibodies to the antigen receptor stimulate a TPK that phosphorylates the ζ chain on tyrosine residues (*5*). Tyrosine phosphorylation of ζ was readily induced by PHA (Fig. 2). However, when T cells were incubated for 10 min with genistein before PHA addition, no phosphorylation of TCR ζ was detected (Fig. 2). At a concentration (30 μg/ml) that completely blocked mitogen-induced TCR ζ phosphorylation (Fig. 2), genistein had no effect on phorbol ester–induced translocation of PKC to the plasma membrane in the same cells and only minimally inhibited (<25%) the phosphorylation of CD3 γ or ε subunits, or both, on serine and threonine residues (*11*).

As tyrosine phosphorylation has been implicated in the regulation of cell growth (*12*), we examined the effects of genistein on mitogen-induced activation of T cells. T cells stimulated with PHA or with OKT3, a monoclonal antibody to CD3, in the presence of genistein failed to undergo the blast transformation typical of activated T cells. Instead, the cells remained as small, round, resting cells (*13*). Likewise, genistein inhibited mitogen-stimulated induction of the interleukin-2 (IL-2) receptor α subunit (p55) and prevented the increase in ornithine decarboxylase activity (Table 1) normally seen during blast transformation (*2, 14*). Moreover, genistein inhibited, in a dose-dependent manner, the PHA- or OKT3-induced proliferation of human T cells (Table 1). This inhibitory effect did not result from drug toxicity; the viability of the cells was not significantly affected by culture in the presence of genistein for 3 days. Furthermore, when human blood lymphocytes were treated with genistein (up to 30 μg/ml) for 20 hours, washed, and then cultured in the presence of OKT3 or PHA, no decrease in proliferation was observed (*13*). These results suggest that genistein is

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