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Immunology

CD5 is phosphorylated on tyrosine after stimulation of the T-cell antigen receptor complex

(T-cell activation/signal transduction/tyrosine kinases/CD72/T-cell–B-cell communication)

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ABSTRACT **When T cells are activated by the T-cell antigen receptor, a number of cellular proteins are phosphorylated on tyrosine. We investigated whether any of these proteins were present on the surface of activated T cells. Using the human leukemic T-cell line Jurkat and normal peripheral blood lymphocytes, we identified a 67-kDa cell surface glycoprotein in anti-phosphotyrosine immunoprecipitates, after treatment of the cells with CD3 antibody. When cell lysates were depleted of CD5 by sequential immunoprecipitation, the 67-kDa phosphotyrosyl polypeptide was no longer precipitated by the phosphotyrosine antibody. Western blot analysis of anti-phosphotyrosine precipitates confirmed that this glycoprotein was CD5. It was possible that CD5 was present in the anti-phosphotyrosine immunoprecipitates due to its physical association with phosphotyrosyl proteins rather than being directly tyrosine-phosphorylated itself. However, Western blot analysis of anti-CD5 immunoprecipitates with phosphotyrosine antibody and phosphoamino acid analysis demonstrated that CD5 was indeed phosphorylated on tyrosine after stimulation of the cells with CD3 antibody and was concomitantly phosphorylated on serine and threonine. Tyrosine phosphorylation of CD5 was maximal 2 min after CD3 stimulation and returned to baseline levels by 60 min. CD5 is expressed on the cell surface of all mature T cells and a small proportion of B lymphocytes and has recently been identified as the ligand for CD72, a receptor present on the surface of all B cells. The present data suggest that tyrosine phosphorylation may be involved in B-cell–T-cell communication.**

Binding of antigenic peptide fragments, presented by major histocompatibility complex molecules, to the T-cell receptor (TCR)–CD3 complex results in the activation of a number of biochemical pathways, culminating in the expression of interleukin 2 and interleukin 2 receptors and subsequent T-cell proliferation (1, 2). Early events in the signal transduction process include the stimulation of both serine and tyrosine kinases, followed by the phosphorylation of many intracellular proteins (3–5). Tyrosine kinase activation is very rapid, being observed 5 sec after engagement of the TCR–CD3 complex (5). Although as many as 20 polypeptides are phosphorylated on tyrosine 1 min after CD3 stimulation, very few of these tyrosine kinase substrates have been identified (6). These include CD3 ζ chain (4), ZAP-70 [a 70-kDa tyrosine phosphoprotein that is associated with ζ chain of the TCR complex (7)], and phospholipase C (PLC)-γ1 (8, 9). The tyrosine phosphorylation and activation of PLC-γ1 result in inositol phospholipid hydrolysis leading to diacylglycerol generation and inositol phosphate release, which in turn stimulate protein kinase C and elevate intracellular Ca^{2+} (3, 10, 11). Activation of PLC can be blocked by genistein (12) and herbimycin A (13), two inhibitors of tyrosine kinases. Thus the tyrosine kinase pathway appears to be able to activate or regulate the PLC pathway.

The importance of tyrosine phosphorylation in T-cell activation is further emphasized by the fact that mutant T cells that do not express the tyrosine phosphatase CD45 fail to proliferate in response to antigen (14). CD45 expression is required for the induction of the early tyrosine phosphorylation of proteins and for the effective coupling of the TCR to the phosphatidylinositol pathway (15). Also, crosslinked CD45 monoclonal antibodies, used alone or in conjunction with CD3 or CD2 antibodies, can inhibit T-cell function (16–18).

As it is clear that the phosphorylation and dephosphorylation of proteins on tyrosine residues play a critical role in T-cell activation, it is essential to identify the proteins that are a target for such enzyme activity. We chose to investigate whether any of these proteins are expressed on the surface of T cells. We report here that the cell surface glycoprotein CD5 is phosphorylated on tyrosine residues after activation of T cells with CD3 antibody and discuss the possible implication of such a modification on the functional interaction of helper T cells with B cells.

MATERIALS AND METHODS

Cells and Antibodies. The human leukemia T-cell line Jurkat (J6) was grown in RPMI 1640 medium containing 5% (vol/vol) fetal calf serum, penicillin (100 units/ml), and streptomycin (50 units/ml). Primary human peripheral blood-derived T cells were prepared by Diana Wallace (Courtauld Institute of Biochemistry, University College Hospital, London) as described (19). The following antibodies were kindly provided by the following individuals: CD3 antibody UCHT1, CD5 antibody UCHT2, and CD45 antibody 2D1, Peter Beverley (University College, London); CD5 antibody OKT1, Martin Glennie and Alison Tutt (Tenovus, Southampton, U.K.); phosphotyrosine antibody 4G10, Brian Druker (Dana–Farber Cancer Institute, Boston); rabbit anti-CD5 peptide serum, raised against the last 20 amino acids at the C terminus, David Mason (John Radcliffe Hospital, Oxford). The monoclonal antibodies were used as purified immunoglobulin, except for UCHT2, which was used as ascitic fluid. Antibodies used for immunoprecipitation were covalently coupled to either protein A- or protein G-Sepharose (Pharmacia) with dimethyl pimelimidate hydrochloride (Sigma) at 2 mg of antibody to 1 ml of packed beads (6).

^{125}I Surface Labeling and Immunoprecipitation. J6 (2×10^7 cells) or primary T (4×10^7 cells) cells were labeled with 0.5–1 mCi of ^{125}I (IMS30, Amersham; 1 Ci = 37 GBq) at their surface for 10 min at room temperature by using the lactoperoxidase method (20). The cells were then washed with ice-cold PBS containing bovine serum albumin (1 mg/ml),

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Abbreviations: TCR, T-cell receptor; PLC, phospholipase C; PVDF, poly(vinylidene difluoride).

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glucose (1 mg/ml), and 1 mM NaI. This wash was repeated using the same buffer without NaI. The cells were finally resuspended at 37°C in 1 ml of RPMI 1640 medium and left to recover for 5 min. In a typical experiment cells were 80% viable after this procedure. The cells were then activated for 1 min with 20 μl of UCHT1 (1 mg/ml) and quickly pelleted in a microcentrifuge, and the reaction was stopped by the addition of 1 ml of lysis buffer [150 mM NaCl/1% Nonidet P-40/10 mM Tris·HCl, pH 7.4/10 mM iodoacetamide/100 μM sodium vanadate/1 mM phenylmethylsulfonyl fluoride/antipain (1 μg/ml)/chymostatin (1 μg/ml)/leupeptin (1 μg/ml)/pepstatin (1 μg/ml)]. The lysates were precleared with two 1-h incubation with protein G-Sepharose (50 μl) and precipitated overnight with the antibodies described in the figure legends. The precipitates were washed five times with lysis buffer, then dissolved by boiling for 5 min in Laemmli sample buffer under reducing conditions (21), and resolved by SDS/PAGE. After electrophoresis the gels were fixed and dried, and the labeled bands were visualized by autoradiography at −70°C using Kodak XAR 5 film.

Immunoprecipitates that were to be deglycosylated by N-Glycanase treatment were washed five times in lysis buffer, followed by one wash in 0.2 M sodium phosphate, pH 8.5/10 mM phenanthroline/1% Nonidet P-40, and then incubated overnight at 37°C with 80 μl of this buffer containing 0.3 unit of N-Glycanase (Genzyme) prior to the addition of 40 μl of 2× Laemmli sample buffer (21).

Western Blot Analysis of Immunoprecipitates. J6 cells (8×10^7 cells) were washed three times with RPMI 1640 medium and then resuspended in 2 ml of this medium. The cells were left to recover for 5 min at 37°C prior to activation with 40 μl of UCHT1 (1 mg/ml), usually for 1–2 min. The reaction was stopped by the addition of 2 ml of lysis buffer to the pelleted cells. Immunoprecipitation and electrophoresis were carried out as described above, except that phosphotyrosyl polypeptides were eluted from the 4G10 beads by two 15-min washes with 40 μl of 100 mM phenyl phosphate in 10 mM Tris·HCl, pH 7.4/0.5% Nonidet P-40/100 μM sodium vanadate. The eluate (80 μl) was combined with 40 μl of 2× sample buffer and boiled for 5 min prior to SDS/PAGE.

After electrophoresis, proteins were transferred electrophoretically to a poly(vinylidene difluoride) (PVDF) membrane (Immobilon P, Millipore) at 0.3 A and 60 V for 16 h. To analyze for phosphotyrosyl polypeptides, blots were incubated with ^{125}I-labeled 4G10 antibody as described (6). When analyzing the blot with the rabbit CD5 anti-peptide serum, the ECL method was used (Amersham).

Biosynthetic ^{32}P Labeling and Phosphoamino Acid Analysis. J6 cells (6×10^7 cells) were washed three times with phosphate-free Dulbecco's modified Eagle's medium and then labeled in this medium containing 5% fetal calf serum and 2 mCi of [^{32}P]orthophosphate (PBS 13; Amersham; final volume, 15 ml) for 4 h. The cells were activated for 2 min with UCHT1 (20 μg/ml) prior to immunoprecipitation with the CD5 antibody OKT1, as described above. After SDS/PAGE, protein was electrophoretically transferred to a PVDF filter and the blot was autoradiographed to determine the position of the CD5 band. Only one major band, which had the mobility expected of CD5, was observed. The band was cut out; the filter was wetted in methanol and washed with water. The bound CD5 was hydrolyzed in 6 M HCl for 4 h at 110°C and the resulting phosphoamino acids were separated and detected as described (6). After the CD5 band had been cut out, the filter was incubated with the rabbit anti-CD5 peptide serum as a final check that the band excised was CD5.

RESULTS

To explore whether any of the polypeptides that were phosphorylated on tyrosine after T-cell activation were cell surface proteins, Jurkat T cells were labeled at their surface with ^{125}I by using the lactoperoxidase procedure and immunoprecipitated with the phosphotyrosine antibody 4G10. No iodinated phosphotyrosyl polypeptides were detected in untreated cells (Fig. 1*A*, lane 1). In contrast, when the cells were stimulated for 1 min with the CD3-specific antibody UCHT1, one prominent diffuse band of ≈67 kDa was detected (Fig. 1*A*, lane 2). This polypeptide was N-Glycanase-sensitive and increased its mobility after digestion to ≈55 kDa (Fig. 1*A*, lane 3). Similarly, an iodinated polypeptide of 67 kDa was detected when this experiment was repeated using CD3-activated peripheral blood T cells (Fig. 1*B*, lane 2). The heavily surfaced labeled polypeptides of low molecular mass (Fig. 1*B*, lane 2) probably represent CD3, due to precipitation with UCHT1 antibody that had not been adequately removed during preclearing.

Because many of the surface proteins of T cells have been identified, it seemed possible that the 67-kDa phosphotyrosyl polypeptide represented a previously designated glycoprotein. Several cell surface glycoproteins of similar size have been described and, of these, CD5 looked most likely, as it had a potential tyrosine phosphorylation site (22). To investigate whether the phosphotyrosyl surface protein was CD5, immunoprecipitates of CD5 and 4G10 were prepared from ^{125}I-surface-labeled Jurkat T cells and resolved on an SDS/polyacrylamide gel. The CD5 and 4G10 antibodies precipitated bands of identical mobility (Fig. 2, compare lanes 1, 5, and 12) that, after N-Glycanase digestion to remove N-linked oligosaccharides, underwent the same increase in mobility to ≈55 kDa (data not shown).

Confirmation that the anti-phosphotyrosine immunoprecipitate contained CD5 was sought in two ways. (*i*) Lysates from CD3-activated ^{125}I-surface-labeled Jurkat cells were depleted of CD5 by sequential immunoprecipitation using either OKT1 (Fig. 2, lanes 1–3) or UCHT2 (Fig. 2, lanes 5–7). The 67-kDa polypeptide was not detected in the CD5-depleted lysates when they were reprecipitated with 4G10

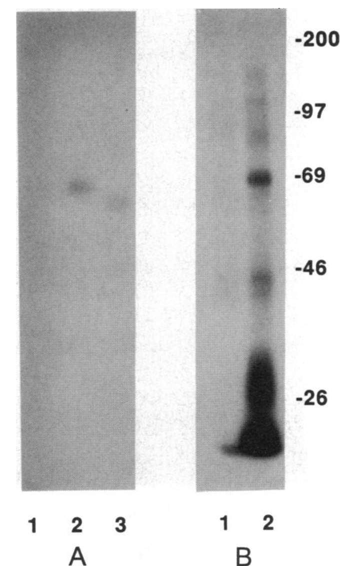

FIG. 1. Identification of surface-labeled phosphotyrosyl protein on CD3-stimulated cells. Jurkat cells (*A*) or primary T cells (*B*) were labeled at their surface with ^{125}I and stimulated with UCHT1 antibody for 0 (lane 1) or 1 min (lanes 2 and 3) prior to immunoprecipitation with 4G10 antibody (10 μg). The immunoprecipitate in lane 3 was treated with N-Glycanase. The phosphotyrosyl polypeptides were separated on a 7–17% gradient polyacrylamide gel by SDS/PAGE and visualized by autoradiography. Positions of prestained molecular mass markers (200 kDa, myosin; 97 kDa, phosphorylase *b*; 69 kDa, bovine serum albumin; 46 kDa, ovalbumin; 26 kDa, carbonic anhydrase) are shown to the right.

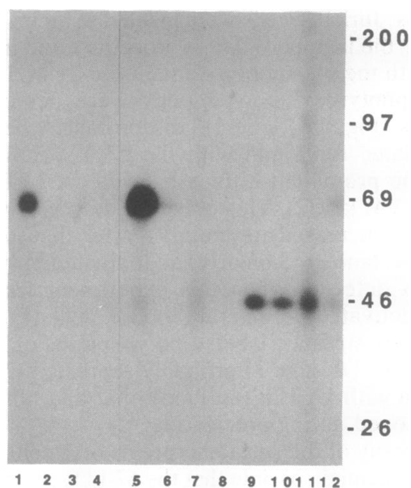

FIG. 2. Immunoprecipitation with 4G10 after CD5 depletion. Jurkat T cells were surface-labeled with ^{125}I, activated with UCHT1 for 90 sec, and then sequentially immunoprecipitated for 16 h, 60 min, and 90 min with CD5 antibodies OKT1 (5 μg; lanes 1–3), UCHT2 (20 μl of ascites fluid; lanes 5–7), or HLA class I antibody W632 (5 μg; lanes 9–11). The lysates that had been depleted of CD5 (lanes 4 and 8) or class I antigen (lane 12) were then precipitated for 120 min with phosphotyrosine antibody 4G10 (10 μg). The proteins were separated by SDS/PAGE (7–17% gradient gel) and visualized by autoradiography.

(Fig. 2, lanes 4 and 8), although it could still be detected in control lysates that had been depleted of major histocompatibility complex class I antigen (Fig. 2, lane 12).

(*ii*) Anti-phosphotyrosine immunoprecipitates prepared from lysates of unlabeled Jurkat cells were assayed for CD5 by immunoblot analysis of the immunoprecipitates with an anti-CD5 peptide serum. As shown in Fig. 3, CD5 was clearly present in the 4G10 immunoprecipitate prepared from cells stimulated with UCHT1 for 2 min (Fig. 3*A*, lane 2) but was barely detectable in immunoprecipitates prepared from untreated cells (Fig. 3*A*, lane 1). When 4G10 immunoprecipitates that had been treated with N-Glycanase were analyzed on an immunoblot with the anti-CD5 peptide serum a 55-kDa band was observed that has the characteristic mobility of CD5 after N-Glycanase treatment (data not shown).

The amount of CD5 that was precipitated by 4G10 was compared densitometrically with that precipitated by CD5 antibody, from the same number of activated ^{125}I-surface-labeled Jurkat cells. The anti-phosphotyrosine precipitate contained between 15 and 20% of the total surface-labeled CD5 (data not shown).

Thus, these experiments suggested that CD5 was tyrosine-phosphorylated in J6 and peripheral blood T cells after TCR–CD3 stimulation. However, it could not be excluded that CD5 was coprecipitated with other phosphotyrosyl polypeptides under the nondenaturing conditions used for cell lysis. To ascertain whether the CD5 antigen was directly tyrosine-phosphorylated, the glycoprotein was immunoprecipitated from unlabeled Jurkat cells and analyzed on an immunoblot with 4G10. A major band with a mobility characteristic of CD5 was detected in anti-CD5 immunoprecipitates prepared from cells activated with UCHT1 for 2 min (Fig. 3*B*, lane 4), whereas anti-CD5 precipitates prepared from untreated cells contained no discernible phosphotyrosine bands (Fig. 3*B*, lane 3). Control immunoprecipitates prepared using either normal mouse IgG (Fig. 3*B*, lanes 1 and 2) or the CD45 antibody, which was of the same class as the CD5 antibody (Fig. 3*B*, lanes 5 and 6), did not contain a band of 67 kDa. These results provide strong supporting evidence that the 67-kDa phosphotyrosine polypeptide is CD5 and that CD5 is directly phosphorylated on tyrosine. To verify the specificity of the immunoblot analysis in this experiment, phosphoserine (Fig. 4*A*, lanes 1 and 2), phosphotyrosine (Fig.

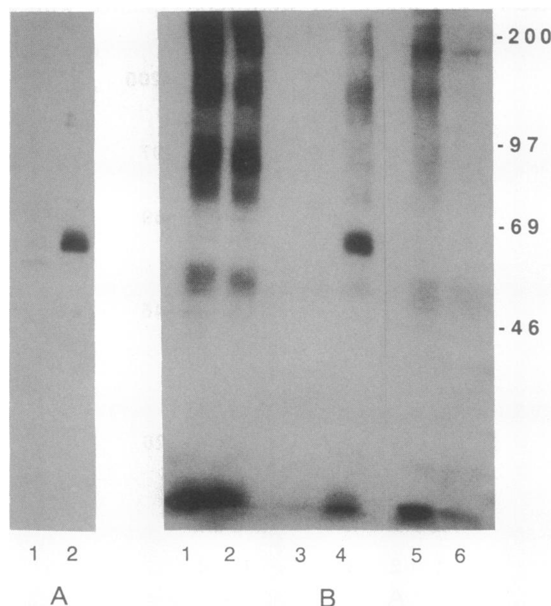

FIG. 3. Detection of CD5 in 4G10 immunoprecipitates. Unlabeled Jurkat cells were activated for 0 min (lanes 1, 3, and 5) or 2 min with UCHT1 (lanes 2, 4, and 6). (*A*) Immunoprecipitation with 4G10 antibody (35 μg; lanes 1 and 2). (*B*) Immunoprecipition with normal mouse immunoglobulin (50 μg; lanes 1 and 2), CD5 antibody OKT1 (50 μg; lanes 3 and 4), or CD45 antibody (50 μg; lanes 5 and 6). The precipitated proteins were electrophoresed by SDS/PAGE on a 10% polyacrylamide gel and transferred to a PVDF filter, which was probed with either CD5 anti-peptide serum (1:100 dilution) (*A*) or ^{125}I-labeled 4G10 (*B*). Bound CD5 antibody was detected by the ECL method in *A* and bound 4G10 was detected by autoradiography in *B*.

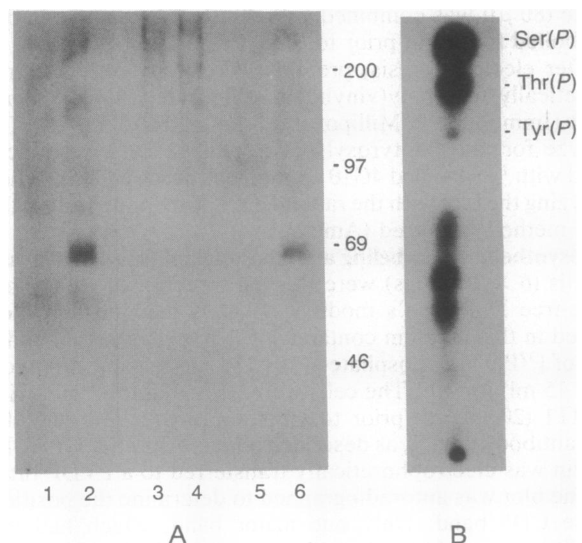

FIG. 4. Direct evidence that CD5 is tyrosine-phosphorylated. (*A*) Unlabeled Jurkat cells were activated for 0 min (lanes 1, 3, and 5) or 2 min (lanes 2, 4, and 6) with UCHT1, lysed, and immunoprecipitated with the CD5 antibody OKT1 (50 μg). The CD5 precipitates were resolved by SDS/PAGE on 10% gels and transferred to a PVDF filter, which was probed with ^{125}I-labeled 4G10 in the presence of 10 mM phosphoserine [Ser(*P*), lanes 1 and 2], phosphotyrosine [Tyr(*P*), lanes 3 and 4], or phosphothreonine [Thr(*P*), lanes 5 and 6]. (*B*) Jurkat cells were *in vivo*-labeled with ^{32}P, activated with UCHT1 for 2 min, and immunoprecipitated with CD5 antibody. Isolated CD5 was hydrolyzed for 4 h with HCl, and the phosphoamino acids were analyzed by electrophoretic TCL.

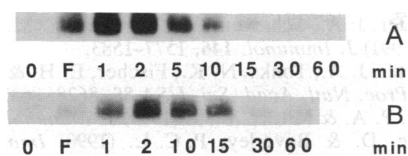

FIG. 5. Time course of CD5 tyrosine phosphorylation. Unlabeled Jurkat cells were activated for the times indicated with UCHT1 antibody. The fastest activation (F) that could be accomplished was the time it took adding the antibody, immediately centrifuging the cells, and adding lysis buffer to stop the reaction (30–40 sec). The lysates were either immunoprecipitated with CD5 antibody and analyzed on an immunoblot with ^{125}I-labeled 4G10 (*A*) or immunoprecipitated with 4G10 antibody and analyzed on an immunoblot with CD5 anti-peptide serum (*B*).

4*A*, lanes 3 and 4), and phosphothreonine (Fig. 4*A*, lanes 5 and 6) were included as inhibitors during the incubation of the PVDF filters with the phosphotyrosine antibody. Only phosphotyrosine competed for the binding of the 4G10 antibody; thus the antibody recognized the 67-kDa polypeptide specifically.

Final confirmation of the tyrosine phosphorylation of CD5 was obtained by phosphoamino acid analysis. CD5 isolated from biosynthetically ^{32}P-labeled Jurkat cells that had been activated with UCHT1 for 2 min was hydrolyzed with HCl for 4 h, and the resulting phosphoamino acids were separated by electrophoretic TLC. Fig. 4*B* indicates that the immunoprecipitated CD5 was phosphorylated on serine, threonine, and tyrosine. Comparison of the patterns of ^{32}P-labeled phosphoamino acids isolated from stimulated and unstimulated cells showed that CD5 was hyperphosphorylated on threonine and probably on serine as well as on tyrosine 2 min after stimulation of T cells with CD3 antibody (data not shown). The large amount of serine and threonine phosphorylation relative to tyrosine in the phosphoamino acid analyses may reflect a higher stoichiometry of phosphorylation of CD5 on the former amino acids. Also the cytoplasmic tail of CD5 contains 11 serine and 4 threonine residues compared with 4 tyrosines. Thus, there are more potential sites for serine and threonine phosphorylation than there are for tyrosine phosphorylation.

Tyrosine phosphorylation is an early and transient response to TCR–CD3 stimulation (6). The time course of CD5 tyrosine phosphorylation was investigated to see whether it followed a similar pattern. Unlabeled Jurkat cells were stimulated for various times with UCHT1 antibody and then either immunoprecipitated with phosphotyrosine antibody or with CD5 antibody. After electrophoretic transfer of the proteins, the PVDF filters were probed with the reciprocal antibody (Fig. 5). Both methods demonstrated that CD5 was rapidly phosphorylated on tyrosine after TCR–CD3 stimulation; the phosphorylation was maximal at 2 min and returned to baseline levels by 1 h. Fig. 5*A* demonstrates the direct tyrosine phosphorylation of CD5, and Fig. 5*B* is a measurement of both tyrosine-phosphorylated CD5 and CD5 that may have been precipitated by its association with other tyrosine-phosphorylated polypeptides.

DISCUSSION

While investigating the cellular locations of substrates for tyrosine kinases after T-cell activation, we identified a 67-kDa phosphotyrosyl glycoprotein expressed on the surface of activated Jurkat cells and primary blood T lymphocytes. This glycoprotein was subsequently identified as CD5.

CD5 is a 67-kDa glycoprotein that is expressed on the surface of all mature T cells and a small proportion of B cells (23, 24). Although CD5 has been reported to be constitutively phosphorylated on serine and threonine residues and hyperphosphorylated on serine residues after phorbol ester stimulation (25–27), to our knowledge, the demonstration of tyrosine phosphorylation of CD5 has not been shown previously. CD5 has a long cytoplasmic domain suggesting a possible role in signal transduction. Consistent with this idea, the observation that the cytoplasmic tail shows 90% amino acid sequence homology between human and mouse CD5 (22, 28) implies that this domain of CD5 has a conserved function. The cytoplasmic tails of both human and mouse CD5 contain four tyrosine residues, one of which is at the membrane interface. Very few recognition sites for tyrosine kinases have been characterized; those so far identified have acidic amino acid residues located near the tyrosine phosphate acceptor site (29). The sequence surrounding Tyr-429 contains two acidic residues (Asp-Asn-Glu-Tyr-Ser-Gln-Pro) and is very similar to the sequence (Asp-Asn-Glu-Tyr-Thr-Ala-Arg) surrounding the tyrosine autophosphorylation site of the src protein tyrosine kinase family. Two members of this family (namely, lck and fyn) have been implicated in T-cell signal transduction. lck tyrosine kinase is noncovalently associated with CD4 and CD8, T-cell surface molecules that bind to major histocompatibility complex class II and I antigens, respectively (30). The association of lck with CD4 and CD8 is required for efficient signal transduction via the TCR complex (31, 32). The fyn tyrosine kinase has been shown to be physically associated with the TCR–CD3 complex (33). Overexpression of fyn in the thymocytes of transgenic mice results in the augmentation of their response to CD3 antibodies (34). Thus the tyrosine kinases lck and fyn are the most likely candidates for the enzyme that phosphorylates CD5 and Tyr-429 is the probable site of their action. However, the sequence surrounding Tyr-463, which is 7 amino acids from the C terminus, contains three aspartic acid residues (Ser-Asp-Ser-Asp-Tyr-Asp-Leu) and should not be overlooked as a possible site for phosphorylation by tyrosine kinases. Further experiments are obviously required to identify the tyrosine phosphorylation site or sites and the kinase or kinases that are involved.

CD5 antibodies have been reported variously to either induce T-cell proliferation directly (35) or potentiate it in response to other stimuli (e.g., CD3 antibody) (36, 37). The CD5 antibody response is dependent on the surface expression of the TCR–CD3 complex. Thus CD5 antibody fails to induce an increase in intracellular Ca^{2+} in TCR β-chain-deficient Jurkat T-cell mutants (35). Similarly, $CD5^+/CD3^-$ leukemic T-cell lines do not respond to CD5 stimulation (36). Conversely, CD5 expression may be a prerequisite for T-cell activation as $CD5^-$ rat T cells, produced by the *in vivo* treatment of the animals with CD5 antibody, do not proliferate in response to alloantigen or mitogen (38). Therefore, several pieces of evidence suggest that the TCR–CD3 complex and CD5 are functionally linked, although the exact nature of this linkage remains unknown.

The ligand for CD5 has recently been identified as CD72, a glycoprotein present on the surface of all B cells (39). CD72 antibodies can stimulate B-cell blastogenesis and act synergistically with immunoglobulin antibodies to promote B-cell proliferation (40, 41). The demonstration that CD5 and CD72 are a pair of interacting receptors on the surface of T and B cells, respectively, has raised the possibility that these receptors may be able to send and receive signals during communication between B and T cells (42). There is evidence suggesting that CD5 may send signals to B cells resulting in their activation. It has been shown that addition of CD5 antibody (OKT1) and autologous $CD4^+$ cells to B cells results in an enhancement of B-cell differentiation (43). Also, perturbation of the CD5 molecule with the CD5 antibody OKT1 in the presence of autologous E^- cells (macrophages) causes the release of molecules that trigger B cells to proliferate and differentiate into immunoglobulin-secreting cells. These data

and the fact that antibodies against CD5 and CD72 are stimulatory suggest that the signals between CD5 on T cells and CD72 on B cells may be bidirectional. It is possible that the tyrosine phosphorylation of CD5 may play a role in the transduction of such signals. From this, it follows that tyrosine phosphorylation of CD5 may be involved in signal transduction not only from the cell surface to the nucleus but also from the inside of the T cell to the outside. Such inside–out transmembrane signaling has been implicated (44) for the integrin family of proteins, where the cytoplasmic tail of the α subunit of glycoprotein gpIIb–IIIa has been shown to control ligand binding affinity and phosphorylation of integrin α_6 has been found to be associated with increased macrophage adhesion to laminin.

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