Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 7099–7103, August 1993
Immunology

β_2-Integrin LFA-1 signaling through phospholipase C-γ1 activation

(lymphocyte function-associated antigen 1/phosphotyrosine/signal transduction/protein-tyrosine kinase)

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Communicated by Seymour J. Klebanoff, April 23, 1993 (received for review February 4, 1993)

ABSTRACT **One of the β_2-integrins found on hematopoietic cells is lymphocyte function-associated antigen 1 (LFA-1), a lymphocyte/myeloid cell-specific receptor that binds to members of the intercellular adhesion molecule (ICAM) family on antigen-presenting cells. Stimulation of LFA-1 with antibodies or purified ICAMs induces augmentation of T-cell antigen receptor (TCR)-directed T-cell responsiveness. In the present study, LFA-1 was shown to be linked to the tyrosine kinase signaling pathway that stimulates tyrosine phosphorylation and activation of phospholipase C-γ1 (PLC-γ1). Integrin β-chain (CD18) crosslinking independently induced downstream mobilization of intracellular Ca^{2+} and potently costimulated TCR-induced Ca^{2+} flux with an increase in both amplitude and kinetics. β_2-Integrin signaling through this pathway was completely inhibited by herbimycin A and was prevented by TCR modulation. Coligation of the TCR via antibody and LFA-1 with a counter-receptor in the form of a soluble ICAM-1/Rg fusion protein resulted in prolonged tyrosine phosphorylation of PLC-γ1. Monoclonal antibodies to both the α chain (CD11a) and the β chain (CD18) of LFA-1 induced Ca^{2+} mobilization to different levels, suggesting epitope specificity for activation potential. In addition to PLC-γ1, tyrosine phosphorylation of an 80-kDa protein substrate was augmented following CD18 crosslinking but was not TCR-dependent. The β_2-integrin LFA-1 on T cells is therefore directly linked to a tyrosine kinase pathway that stimulates signaling by phosphatidylinositol-specific PLC-γ1.**

Integrins expressed on hematopoietic cells have been associated functionally with both cellular adhesion and homing responses (1, 2). Ligands for this class of receptors are extracellular matrix proteins, including fibronectin, collagen, and fibrinogen (1–3), or, alternatively, counter-receptors on apposing cells (1, 2). One integrin receptor expressed on T lymphocytes which binds to its counter-receptors on antigen-presenting cells (APCs) is lymphocyte function-associated antigen 1 (LFA-1), a β_2-integrin. Functional activity of LFA-1 requires association of the α chain (CD11a) with the β chain (CD18) to form a heterodimeric complex that directly contacts intercellular adhesion molecule (ICAM)-family molecules on APCs (e.g., CD54) (1, 4–10). Such interaction induces priming of resting $CD4^+$ T cells for stimulation through T-cell antigen receptors (TCRs) (11–13) or is transiently stimulated following TCR crosslinking (5) and may augment TCR-directed T-cell activation (12, 13).

The specific signal-transduction pathways through which integrins mediate their effects have only recently begun to emerge. Several reports have indicated that stimulation of the β_1-family of integrins, including $\alpha_4\beta_1$, results in tyrosine phosphorylation of proteins in both fibroblasts (14, 15) and T cells (16). Studies have begun to identify the substrates of activated tyrosine kinases in T cells. For example, following stimulation of the TCR, Ca^{2+} is mobilized as a function of the breakdown of phosphatidylinositol bisphosphates into the second messenger inositol trisphosphate (17). The γ1 isoform of phospholipase C (PLC-γ1) is potently activated by tyrosine phosphorylation (18) in T cells stimulated through TCR/CD3, CD2, and CD4 (19–25) and is the key phospholipase responsible for inositol trisphosphate and diacylglycerol generation in the phosphatidylinositol pathway (19) leading ultimately to cytokine gene expression and T-cell expansion (17). Here we demonstrate that a β_2-integrin (LFA-1) induces activation of this tyrosine kinase–phosphatidylinositol pathway through specific activation of PLC-γ1.

MATERIALS AND METHODS

Antibodies and Reagents. Monoclonal antibodies (mAbs) directed against hematopoietic cell surface receptors were gifts, or were obtained from the American Type Culture Collection, Becton Dickinson Monoclonal Center (Mountain View, CA), and AMAC (Westbrook, ME), or have been described (26–30). ICAM-1/Rg and VCAM-1/Rg fusion proteins were prepared as described (12, 31). Phytohemagglutinin (PHA)-P was obtained from Wellcome Diagnostics, herbimycin A from Sigma, and $F(ab')_2$ goat anti-mouse IgG Fc from Cappel. Antiserum to PLC-γ1 was generated as described (25) and mAb to PLC-γ1 was purchased from Upstate Biotechnology (Lake Placid, NY). Affinity-purified rabbit anti-phosphotyrosine (anti-pTyr) was generated as described (32), and anti-pTyr mAb 6G9 was described previously (33).

Isolation of $CD4^+$ T Cells and Generation of Activated T Cells. Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated on Ficoll/Hypaque density gradients. Resting $CD4^+$ T cells were separated from PBMCs by immunomagnetic negative selection using M-450 Dynabeads (Dynal, Great Neck, NY) (12, 13). These cells did not proliferate in response to mitogenic concentrations of PHA-P (10 μg/ml) or soluble anti-CD3 mAb (G19-4) at 100 ng/ml in the absence of APCs. DRw6-primed $CD4^+$ T cells (antigen-primed cells) were prepared as described (12, 13). For the generation of T-cell blasts, PBMCs were incubated for 3 days with PHA-P (1 μg/ml) and then further cultured for 2–3 days without additional PHA-P.

Cytoplasmic Ca^{2+} Measurements. T cells were incubated with primary mAb for 10 min, washed, and then incubated with affinity-purified rabbit anti-mouse IgG. Intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ was measured with indo-1 (Molecular Probes) and a model 50 HH/2150 flow cytometer (Ortho Instruments) (34). There are 100 data points on the x axis (time) for each flow cytometric analysis.

Immunoprecipitation and Western Immunoblotting. Immunoprecipitates of PLC-γ1, CD6, Vav, TCR ζ chain, or pTyr-

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Abbreviations: APC, antigen-presenting cell; LFA, lymphocyte function-associated antigen; ICAM, intercellular adhesion molecule; PLC, phospholipase C; TCR, T-cell antigen receptor; mAb, monoclonal antibody; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; PHA, phytohemagglutinin; pTyr, phosphotyrosine.

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containing proteins were prepared by lysis of ≈2 × 10^7 cells (≈2 mg of protein) in 0.5 ml of modified RIPA buffer containing 1% (vol/vol) Nonidet P-40, 0.25% (wt/vol) sodium deoxycholate, 150 mM NaCl, and 50 mM Tris·HCl, pH 7.5, supplemented with proteinase and phosphatase inhibitors (25), followed by incubation of cellular lysates with 5 μg of protein A-purified mAb or 10 μl of antiserum for 2 hr at 4°C. Immunocomplexes were recovered by addition of 50 μl of protein A-Sepharose beads (Pharmacia) or beads that had been preincubated with 5 μg of affinity-purified rabbit anti-mouse IgG (Jackson ImmunoResearch). The beads were processed as described (25) and immunoprecipitates were immunoblotted with either rabbit anti-pTyr (2 μg/ml) followed by ^{125}I-labeled protein A or mAb to PLC-γ1 followed by ^{125}I-labeled anti-mouse IgG (24, 25).

RESULTS

CD18 Ligation Induces Intracellular Ca^{2+} Mobilization. We investigated the possibility that crosslinking LFA-1 with anti-CD18 mAb could both stimulate Ca^{2+} mobilization and augment TCR-driven signals, since costimulation through LFA-1 with ICAM-1 augments TCR-directed proliferation (13). mAb to CD18 (60.3) induced increases in $[Ca^{2+}]_i$ in both resting and antigen-activated $CD4^+$ human T cells (Fig. 1). Clearly, CD18 ligation alone induced potent Ca^{2+} mobilization. In addition, coaggregation of TCR with CD18 resulted in both an increase in the amplitude of induced $[Ca^{2+}]_i$ changes and an acceleration in kinetics. The kinetics of Ca^{2+} mobilization differed between TCR ligation alone and those of CD18 crosslinking alone. Further, the kinetics of Ca^{2+} signals were delayed in antigen-primed cells. These data suggest that specific alterations occur in coupling of receptors to components of intracellular activation pathways and that such coupling is regulated following stimulation with antigen. Of additional interest, virtually all of the resting cells responded to antibody crosslinking, whereas only 50–60% of the antigen-primed T cells were responsive (Fig. 1 *Lower*). Finally, crosslinking of the β_1-integrin CD29 with mAb 4B4, K-20, or P4C10 (4B4 data shown) did not stimulate Ca^{2+} mobilization in either resting or activated T cells. In $[Ca^{2+}]_i$ experiments, PHA-stimulated T-cell blasts gave results comparable to those observed with antigen-activated $CD4^+$ T-cells (data not shown). For subsequent experiments requiring large numbers of T cells, such as immunoprecipitations, PHA blasts were used. Taken together, these results demonstrate that the β_2-integrin LFA-1 on $CD4^+$ T cells can independently stimulate signaling functions that synergize with TCR-induced signals.

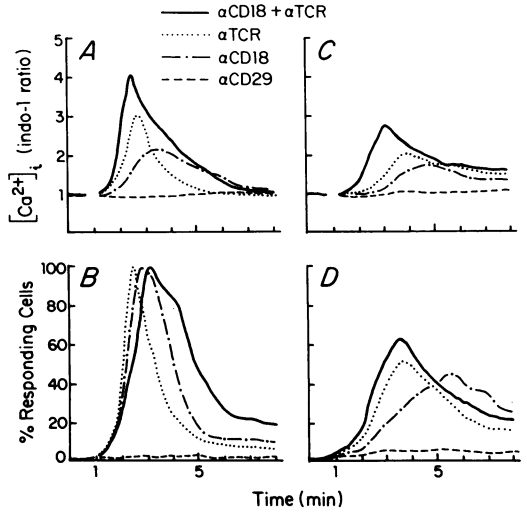

FIG. 1. Mobilization of intracellular Ca^{2+} induced by CD18 ligation. (*A*) Resting $CD4^+$ T cells were incubated with anti-CD29 (4B4), anti-CD18 (60.3), and/or anti-TCR (WT31) for 15 min, washed, and then crosslinked with $F(ab')_2$ goat anti-mouse IgG Fc. α, Anti. (*B*) Percent responding cells from *A*. (*C*) DRw6-primed $CD4^+$ T cells were treated exactly as in *A*. (*D*) Percent responding cells from *C*.

Tyrosine Phosphorylation of PLC-γ1 Following LFA-1 Crosslinking. Stimulation of the TCR by ligation with mAb induces tyrosine phosphorylation of PLC-γ1 (19–25). Further, Ca^{2+} mobilization strongly correlates with activation of PLC-γ1 by stimulation of T-cell surface molecules CD2, CD3, and CD4 (24, 25). We sought to examine whether integrin-induced Ca^{2+} mobilization was linked to the activation of PLC-γ1 by tyrosine phosphorylation. CD18 ligation induced tyrosine phosphorylation of PLC-γ1 (Fig. 2*A*) consistent with the rise in $[Ca^{2+}]_i$ observed in Fig. 1. In addition, CD18 crosslinking resulted in tyrosine phosphorylation of the coimmunoprecipitated pp35/36 protein previously described in complex with PLC-γ1 (24, 25) through the Src-homology 2 (SH2) domains of PLC-γ1 (35). Ligation of TCR and CD18 resulted in increased PLC-γ1 activation (Fig. 2*B*), without any effect on the steady-state level of PLC-γ1 in the cell (Fig. 2*C*). Finally, tyrosine phosphorylation of an additional unidentified protein of 125 kDa coprecipitating with PLC-γ1 was increased 2.5-fold (as judged by laser densitometry) following ligation of TCR and CD18 versus ligation of TCR alone (Fig. 2*B*). In contrast, virtually no increase in pp35/36 tyrosine phosphorylation was observed concomitantly, suggesting that the PLC-γ1 protein complex is differentially regulated following specific receptor ligation. These results demonstrate the contribution of tyrosine kinase activity to LFA-1 signaling in T-cells through phosphorylation of PLC-γ1, a key component in the TCR signal-transduction pathway.

Both Ca^{2+} mobilization and inositolphospholipid hydrolysis in $CD4^+$ T cells stimulated with immobilized anti-TCR/CD3 and purified ICAM-1 were prolonged in comparison to anti-TCR/CD3 activation alone (36). To determine whether ICAM-1 could affect anti-TCR-induced PLC-γ1 activation, T cells were stimulated with immobilized anti-TCR, immobilized anti-TCR plus immobilized ICAM-1/Rg fusion protein (containing the ICAM-1 extracellular domain fused to the

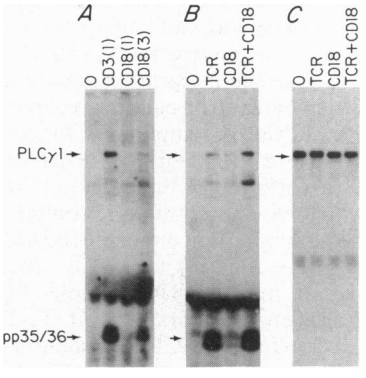

FIG. 2. Tyrosine phosphorylation and expression of PLC-γ1 upon β_2-integrin stimulation. (*A*) T-cell blasts were incubated with mAb to CD3 (G19-4) or CD18 (60.3) for 10 min, washed, and then crosslinked with $F(ab')_2$ goat anti-mouse IgG Fc for 1 or 3 min as indicated in parentheses. Immunoprecipitates of PLC-γ1 from cell lysates were immunoblotted with anti-pTyr. 0, Unstimulated cells. PLC-γ1 and tyrosine-phosphorylated proteins pp35/36 coimmunoprecipitating with PLC-γ1 are indicated at left. Bands at ≈50 kDa are IgG heavy chain. (*B*) Augmentation of PLC-γ1 tyrosine phosphorylation by coligation of CD18 (60.3) and TCR (WT31). Immunoprecipitates of PLC-γ1 were immunoblotted with anti-pTyr. (*C*) Identical immunoprecipitates prepared as in *B* were immunoblotted with mAb to PLC-γ1.

human IgG Fc domain), or immobilized anti-TCR plus immobilized VCAM-1/Rg. PLC-γ1 was immunoprecipitated from cell lysates following stimulation for various times and immunoblotted with anti-pTyr, and the PLC-γ1 band was analyzed by densitometry. ICAM-1 augmented PLC-γ1 tyrosine phosphorylation, and its activation was prolonged compared with anti-TCR alone or in combination with VCAM-1 (Table 1). These data further indicate that LFA-1 is coupled to intracellular signaling pathways involving tyrosine kinase stimulation leading to PLC-γ1 activation.

To further assess the link between CD18 signaling and tyrosine kinase activity, T cells were treated with the benzoquinonoid ansamycin antibiotic herbimycin A, a potent tyrosine kinase inhibitor (37, 38). Both CD18-stimulated and TCR-induced PLC-γ1 tyrosine phosphorylation were ablated by herbimycin A, without a concomitant reduction in expression of PLC-γ1 (Fig. 3*A*).

TCR Modulation Prevents LFA-1 Signaling. Pretreatment of T cells with anti-TCR/CD3 results in a refractory state, in which the T cells fail to signal through CD2, CD3, or CD28 (39–41). Ca^{2+} stores are not depleted following anti-CD3 desensitization, and such T cells will show increased $[Ca^{2+}]_i$ following ionophore treatment (39). T cells were treated with anti-TCR to investigate the dependence of CD18 signaling on the TCR. β_2-Integrin-induced Ca^{2+} mobilization was profoundly diminished following TCR modulation by anti-TCR pretreatment for 1 hr. In addition, <20% of T cells responded to either TCR or CD18 ligation following anti-TCR treatment (Fig. 3*B Lower*). These data indicate that CD18 signaling through PLC-γ1-mediated Ca^{2+} mobilization appears to require surface expression of TCR molecules.

Linkage of LFA-1 α and β Chains to the Signaling Pathway. Although antibodies to the β chain of LFA-1 can independently signal in this system, we addressed whether ligation of the α chain (CD11a) could also induce changes in $[Ca^{2+}]_i$. mAbs to CD11a were compared with mAbs to CD18 for effects on mobilization of intracellular Ca^{2+} (Table 2). Three different mAbs to CD11a induced low levels of Ca^{2+} in 45–50% of resting $CD4^+$ T cells. Equally, the CD18-specific mAb BL5 induced similar increases in $[Ca^{2+}]_i$, whereas 60.3 had the most potent stimulatory activity on virtually all T cells. Likewise, ligation of CD11a and TCR stimulated modest increases in $[Ca^{2+}]_i$ that appeared additive, while that observed with 60.3 and anti-TCR was often synergistic (see also Fig. 1). In addition, mAbs that induced responsiveness in 45–50% of $CD4^+$ T cells stimulated Ca^{2+} mobilization in $CD4^+/RA^+$ cells but not $CD4^+/RO^+$ T cells (data not shown). We do not know whether LFA-1 epitope differences occur among $CD4^+$ T-cell subsets or whether CD45 isoforms affect LFA-1 signaling capacity. These results indicate that both the α chain and the β chain of LFA-1 mediate signaling activity.

Table 1. Prolonged activation of PLC-γ1 following coligation of TCR and ICAM-1

Stimulation	Time, min	PLC-γ1 activation
Anti-TCR	10	32
	30	19
Anti-TCR + ICAM-1/Rg	10	43
	30	47
Anti-TCR + VCAM-1/Rg	10	33
	30	15

T-cell blasts were stimulated with immobilized anti-TCR mAb alone (10 μg/ml) or in combination with immobilized ICAM-1/Rg or VCAM-1/Rg (10 μg/ml) for 10 and 30 min. PLC-γ1 activation was determined by tyrosine phosphorylation. PLC-γ1 was immunoprecipitated from cell lysates following stimulation and immunoblotted with anti-pTyr and detected by autoradiography as in Fig. 2. The film was scanned on an LKB Ultroscan XL laser densitometer and the band corresponding to PLC-γ1 was analyzed. PLC-γ1 from unstimulated cells was unphosphorylated on tyrosine and provided the baseline, and activation is indicated in arbitrary units.

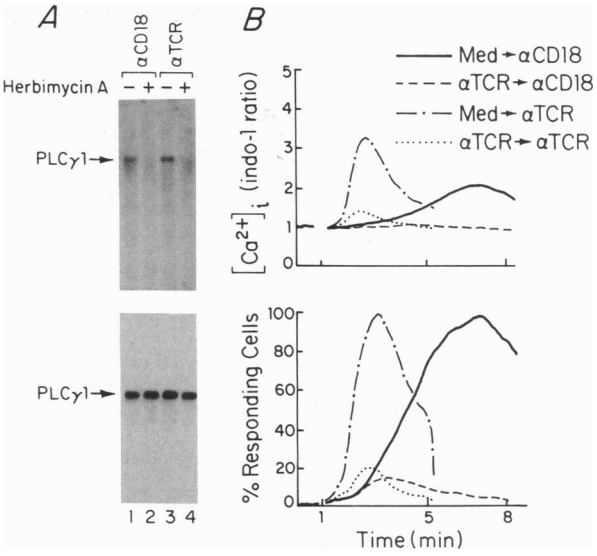

FIG. 3. Dependence of CD18 signaling on tyrosine kinases and TCR. (*A*) T-cell blasts were incubated in the absence (−) or presence (+) of herbimycin A (1 μg/ml) for 16 hr and then stimulated with either anti-CD18 (60.3) or anti-TCR (WT31). Immunoprecipitates of PLC-γ1 were then prepared and immunoblotted with anti-pTyr (*Upper*) or anti-PLC-γ1 (*Lower*). (*B*) CD18-induced $[Ca^{2+}]_i$ was measured after treatment of T-cell blasts with culture medium (Med) or anti-TCR mAb (αTCR, 20 μg/ml). $[Ca^{2+}]_i$ measurements are indicated by indo-1 ratio (*Upper*) and percent responding cells (*Lower*).

Tyrosine Phosphorylation of the Protein Substrate pp80 by CD18 Crosslinking. Ligation of several T-cell surface receptors results in the tyrosine phosphorylation of intracellular protein substrates (42). Since CD18-induced PLC-γ1 tyrosine phosphorylation was readily observed in T cells, it was of interest to address whether additional proteins were phosphorylated on tyrosine following CD18 ligation. T cells were stimulated with anti-CD18, anti-CD3, or both and total pTyr-containing proteins were immunoprecipitated with anti-pTyr mAb 6G9 and immunoblotted with rabbit anti-pTyr (Fig. 4*A*). CD18 ligation induced an increase in one major 80-kDa pTyr-containing protein (pp80) by this method; however, PLC-γ1 could not be visualized without prior immunoprecipitation with its specific antibody. In contrast, anti-CD3 also induced an increase in the pTyr content of the 80-kDa protein and that of several additional species. Ligation of CD3 and CD18 appeared qualitatively similar to CD3 crosslinking alone. Ligation of CD3 and CD4 resulted in an increase in the pTyr content of several proteins, including pp80. To address whether the CD18-induced tyrosine kinase activity was specific and not exclusively the result of TCR-directed signaling, three additional known TCR/CD3-stimulated tyrosine kinase substrates were assayed. Ligation of CD3 resulted in increased tyrosine phosphorylation of CD6 (130 kDa) (43), Vav (95 kDa) (44), and the TCR/CD3 ζ chain (21 kDa) (45) (Fig. 4*B*), and these increases were all augmented by CD3 plus CD4 coaggregation as previously observed for PLC-γ1. In contrast, crosslinking of CD18 did not alter the extent of tyrosine phosphorylation of these three protein substrates, nor were any increases observed following CD3 plus CD18 receptor clustering. Furthermore, limited CD3 stimulation did not synergize with CD18 induction of pp80 (Fig. 4*C*), suggesting that the 80-kDa substrate was phosphorylated following ligation of β_2-integrins not associated with TCR. These results indicate that CD18 is linked to tyrosine kinases that phosphorylate both PLC-γ1 and the

Table 2. Mobilization of Ca^{2+} in resting $CD4^+$ T cells by mAb directed to LFA-1

		Peak $[Ca^{2+}]_i$, nM (% responding cells)			
		Exp. 1		Exp. 2	
mAb	Specificity	IgG	Anti-TCR	IgG	Anti-TCR
IgG	—	131	945 (>95)	131	835 (>95)
25-3	CD11a	170 (50)	945 (>95)	185 (50)	ND
TS1/22	CD11a	205 (50)	1100 (>95)	185 (45)	945 (>95)
GR53	CD11a	195 (45)	ND	195 (50)	1000 (>95)
BL5	CD18	225 (50)	1150 (>95)	170 (50)	1100 (>95)
60.3	CD18	480 (>95)	1820 (>95)	480 (>95)	1346 (>95)

Fresh resting $CD4^+$ T cells were treated for 30 min with either nonbinding control mouse IgG (10 μg/ml) or mAb (10 μg/ml) directed to specific chains of the CD11a/CD18 complex in the presence of mouse IgG [nonbinding control (1 μg/ml)] or anti-TCR mAb WT31 (1 μg/ml). T cells were then washed to remove any unbound antibody and examined for $[Ca^{2+}]_i$ response upon crosslinking with $F(ab')_2$ goat anti-mouse IgG Fc (20 μg/ml). ND, not done.

pp80 substrate and that CD18-induced signaling is not strictly an indirect stimulation of the TCR.

DISCUSSION

One functional consequence of T-cell integrin interaction with extracellular matrix proteins or counter-receptors on APCs is adhesion. However, cellular activation generally occurs concomitantly with adhesion events (46, 47). For example, stimulation of TCR/CD3 with antibody or specific antigen activates T cells, whereas integrin coligation with TCR/CD3 results in augmented cellular proliferation (2, 12, 13, 48, 49). Such observations suggest that integrins are linked to intracellular signaling pathways. Here we have demonstrated a link between LFA-1 and the tyrosine kinase–phosphatidylinositol pathway in T cells that activates PLC-γ1 through tyrosine phosphorylation.

Previous signaling studies have focused on the ability of LFA-1 coligation with TCR to prolong increased $[Ca^{2+}]_i$ and inositolphospholipid hydrolysis after the initial spike (36, 50),

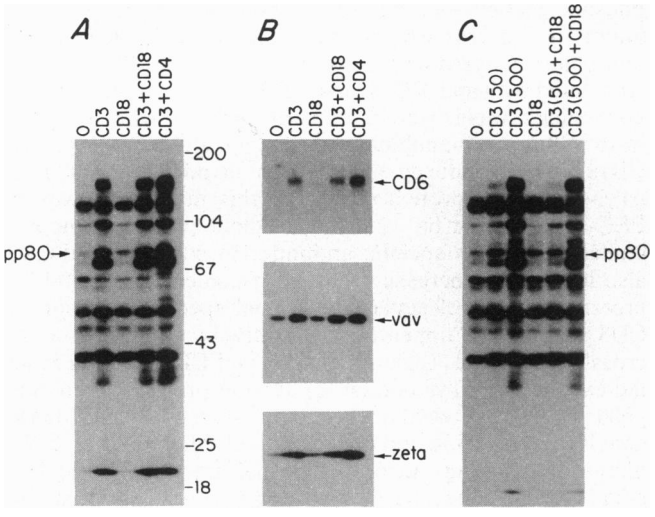

FIG. 4. Tyrosine phosphorylation signals upon β_2-integrin ligation. (*A*) T-cell blasts were stimulated with anti-CD18 (60.3), anti-CD3 (G19-4), and anti-CD4 (G17-2) (10 μg/ml) and then crosslinked as described in Fig. 2. Total pTyr-containing proteins were analyzed following immunoprecipitation with mAb 6G9 and immunoblotting with rabbit anti-pTyr. The pTyr-containing 80-kDa protein substrate pp80 is indicated at left. Size markers are in kilodaltons at right. (*B*) Immunoprecipitates of CD6 (130 kDa), Vav (95 kDa) and TCR ζ chain (21 kDa) were prepared from a portion of the lysates used in *A* and were immunoblotted with anti-pTyr. (*C*) Cells were stimulated essentially as in *A* except that anti-CD3 was used at 50 or 500 ng/ml as indicated in parentheses. Anti-pTyr immunoprecipitates were prepared with mAb 6G9 and were immunoblotted with rabbit anti-pTyr.

and we have extended those observations to include PLC-γ1 as a signaling component of that pathway. However, in those previous investigations, anti-CD18 mAb 60.3 or ICAM-1 did not by itself induce signaling (36, 50), whereas Pardi *et al.* (48) demonstrated low levels of Ca^{2+} mobilization with anti-CD11a in T-cell subsets, and others have shown CD11b/CD18 (Mac-1)-induced Ca^{2+} oscillations in human neutrophils (51, 52). We suggest that integrin crosslinking stoichiometry may play a role in the extent to which signaling events may be measured. Specifically, in resting $CD4^+$ T cells, where high levels of unengaged integrins are expressed, stimulation of LFA-1 induced high $[Ca^{2+}]_i$. In antigen-primed cells the peak levels were lower, potentially reflecting preactivated integrins that no longer responded to stimuli, possibly a function of blocked linkage to the TCR signaling apparatus or maturational differences in components that affect integrin signaling.

In addition to PLC-γ1 and pp80, other proteins become tyrosine-phosphorylated through integrin-induced activation of tyrosine kinases. Specifically, crosslinking of the β_1-integrin CD29 on T cells with antibody resulted in the tyrosine phosphorylation of a 105-kDa protein substrate (16). This protein has not been identified and does not correspond to pp80. Indeed, LFA-1 ligation did not induce the 105-kDa protein substrate (16). Although the identity of pp80 is unknown, it may correspond to the 81-kDa cytoskeletal protein ezrin (53). Of particular interest is the observation in fibroblasts that stimulation of β_1-integrins with antibody or fibronectin leads to the tyrosine phosphorylation of a 125-kDa protein, recently identified as a novel tyrosine kinase coined $pp125^{FAK}$ or FadK (focal adhesion kinase) (54, 55). The data indicate that $pp125^{FAK}$ colocalizes with focal adhesions where integrins cluster (54, 55), suggesting that integrins may activate this tyrosine kinase to phosphorylate cytoskeletal components. β_1-Integrin ligation did not signal Ca^{2+} mobilization in our system, and $pp125^{FAK}$ activation did not occur following β_2-integrin stimulation (data not shown). Thus, β_1- and β_2-integrins most likely mediate cellular responses through stimulation of different effector molecules, either by activation of kinases linked to the cytoskeletal matrix or by stimulation of kinases in the PLC-γ1 signaling pathway.

The identification of PLC-γ1 as a common response element in the signal-transduction pathway for TCR/CD3 and the coreceptors CD2, CD4, and LFA-1 indicates that this protein is a key component for driving T-cell activation. However, the various T-cell coreceptors may direct signals with different outcomes. For example, tyrosine phosphorylation of membrane-associated molecules such as CD5 and CD6 occurs upon stimulation of TCR/CD3 but not after ligation of CD2 or CD4 (43). In contrast, CD2 and CD4 coreceptors can independently induce tyrosine phosphorylation of the substrate PLC-γ1 (25, 56). Additionally, costim-

ulation of resting CD4+ T-cells with antibody to TCR/CD3 and a soluble form of the counter-receptor to LFA-1 (ICAM-1) results in stimulation of interleukin 2 (IL-2) production, whereas such IL-2 synthesis is not detected when antigen-primed CD4+ T-cells are assayed (13). In contrast, CD2 costimulation (with soluble LFA-3) induces IL-2 production in antigen-activated but not resting CD4+ T cells (13). Thus, the stimulatory pathways through which T-cell coreceptors (i.e., LFA-1) function differ, in that they are probably involved in differential signaling during distinct stages of T-cell maturation.

We thank Drs. E. Engleman, W. Tax, C. Morimoto, F. Garrido, J. Sancho, C. Terhorst, and M. Barbacid for antibodies; K. Klussman and T. Tsu for technical assistance; D. Hewgill for flow cytometry assistance; M. West for help in manuscript preparation; and Dr. R. Mittler for comments on the manuscript.

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