Mod Rheumatol (2000) 10:8–15

REVIEW ARTICLE

Chikao Morimoto · Hiroshi Kobayashi · Rikako Nishijima
Hirotoshi Tanaka · Satoshi Iwata

Role of the β1 integrin molecule in T-cell activation and migration

Received: August 24, 1999 / Accepted: August 31, 1999

Abstract β1 integrins play crucial roles in a variety of cell processes such as adhesion, migration, proliferation, and differentiation of lymphocytes. To understand the molecular mechanisms of these various biological effects, it is particularly important to analyze cell signaling through the β1 integrins. Our previous study showed that PLC-γ, pp125FAK (focal adhesion kinase), pp105, paxillin, p59fyn, p56lck, and ERK1/2 are phosphorylated in their tyrosine residues upon engagement of β1 integrins. We identified pp105 as Cas (Crk-associated substrate)-related protein and successfully cloned its cDNA. pp105 is a Cas homologue predominantly expressed in the cells of lymphoid lineage, which led us to designate it Cas-L. Like p130Cas, Cas-L contains a single SH3 domain and multiple SH2-binding sites (YXXP motif), which are suggested to bind SH2 domains of Crk, Nck, and SHPTP2. Subsequent studies revealed that pp125FAK binds Cas-L on its SH3 domain and phosphorylates its tyrosine residues upon β1 integrin stimulation. Since Cas-L is preferentially expressed in lymphocytes, it is conceivable that Cas-L plays an important role in lymphocyte-specific signals. We have shown that Cas-L is involved in the T-cell receptor (TCR)/CD3 signaling pathway as well as the β1 integrin signaling pathway. Cas-L is transiently phosphorylated following CD3 crosslinking and tyrosine-phosphorylated Cas-L binds to Crk and C3G. Furthermore, a Cas-L mutant (Cas-LΔSH3), which lacks the binding site for FAK, is still tyrosine-phosphorylated upon CD3 crosslinking but not upon β1 integrin crosslinking, suggesting that FAK is not involved in CD3-dependent Cas-L phosphorylation. Finally, we have identified a crucial role of Cas-L in β1 integrin-mediated T-cell co-stimulation. We have found that this co-stimulatory pathway is impaired in the Jurkat T-cell line, and that the expression level of Cas-L is reduced in the Jurkat cells compared to peripheral T-cells. The transfection of Cas-L cDNA into Jurkat cells restored the β1 integrin-mediated co-stimulation, while the transfection of Cas-LΔSH3 mutant failed to do so, which contrasts with the case of CD3-mediated signaling. These results indicate that Cas-L plays a key role, through the association and phosphorylation by FAK, in β1 integrin-mediated T-cell co-stimulation. Moreover, tyrosine phosphorylation of Cas-L is critical for T-cell receptor and β1 integrin-induced T-lymphocyte migration. Taken together, Cas-L might be the bi-modal docking protein which assembles the signals through β1 integrins and TCR/CD3, and which participates in a variety of T-cell functions.

Key words β1-integrins · VLA proteins · Tyrosine phosphorylation · p130Cas (Crk-associated substrate) · pp105Cas-L · pp125FAK (focal adhesion kinase) · Paxillin

C. Morimoto (✉) · H. Kobayashi · R. Nishijima · H. Tanaka
Department of Clinical Immunology and AIDS Research Center, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
Tel. +81-3-5449-5546; Fax +81-3-5449-5448
e-mail: morimoto@ims.u-tokyo.ac.jp

C. Morimoto · S. Iwata
Division of Tumor Immunology, Dana–Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, MA 02115, USA

Introduction

VLA (very late activation antigen) constitutes the β1 subfamily of integrin adhesion receptors, defined by at least nine α-chains, that share a non-covalently linked common β1 chain (CD29).[1] The VLA mainly functions as cell surface receptors mediating cell-to-cell and cell-to-ECM (extracellular matrix) adhesion. Among those β1 integrins, VLA-4 and 5 (α4β1 and α5β1) are the major subclasses of β1 integrins expressed on T-lymophocytes. As well as their roles in cell adhesion, recent studies have clearly shown that VLA receptors transduce signals in a wide variety of cells.[2–4] In T-cells in particular, we have shown that the binding of T-cells with ECM through VLA-4 and 5 provides co-stimulatory signals for TCR/CD3-mediated T-cell proliferation.[5–9] It is also reported that β1 integrins are involved in the differentiation of T-cells and B-cells through their inter-

action with FN (fibronectin) expressed on stromal cells in thymus or bone marrow.[10,11]

Although most members of the VLA family are involved in cell–ECM interactions, only VLA-4 has conclusively been shown to participate in both cell–ECM and cell–cell adhesive interactions. In particular, VLA-4 has been shown to serve as a receptor for an Arg–Gly–Asp (RGD)-independent site of FN, namely CS1, as well as for the cell surface molecule, vascular cell adhesion molecule-1(VCAM-1), a member of the Ig superfamily expressed on cytokine-activated endothelial cells. Moreover, accumulating evidences suggest that VLA-4 integrin-dependent adhesion pathways are critical points of intervention in several inflammatory and autoimmune diseases. CD4+CD29/VLA+T-cells are enriched in synovial fluid lymphocytes[12,13] from patients with RA and other inflammatory joint diseases, and also in inflammatory lesions of the brain in MS.[14] VLA-1- and VLA-4-positive T-cells have been shown to localize to sites of inflammation in RA.[15,16] Moreover, the numbers of VLA-4-positive T-cells have been shown to be increased in SLE-associated vasculitis.[17] The endothelial ligand for VLA-4 is VCAM-1, and there is increased expression of endothelial adhesion molecules (ICAM-1 and VCAM-1) in the microvasculature of rheumatoid, but not osteoarthritic, synovium.[18] Increased expression of ICAM-1 and VCAM-1 in the synovium is expected because of the presence of high concentrations of inflammatory cytokines. Thus, the interaction of β1 integrin and its ligand plays an important role in cell migration and in triggering the inflammatory response at the site where the cells migrated. In this review, we will focus on VLA-4-mediated signal transduction through pp105Cas-L, a novel docking protein in lymphocytes, which has recently been cloned in our laboratory and shown to be a homologue of p130Cas (Crk-associated substrate).

Tyrosine phosphorylation in T-cells through the ligation of VLA-4

In an earlier study,[19] we demonstrated that liquid-phase cross-linking of VLA-4 by an anti-VLA-4 mAb, 8F2, and by an anti-CD29 mAb, 4B4, can induce tyrosine phosphorylation of a 105-kDa protein. On the other hand, the solid-phase cross-linking of VLA-4 using 4B4 and 3G6, another anti-VLA-4 mAb recognizing different epitope from 8F2, induced various tyrosine-phosphorylated proteins migrating at 140-, 120-, 110–105-, 80–70-, 60–55-, 50-, and 45-kDa on an SDS-PAGE.[20] In contrast, as is the case with the liquid-phase cross-linking of VLA-4, the solid-phase cross-linking using the higher concentration of 8F2 (20 μg/ml) induced several weakly tyrosine-phosphorylated proteins such as 140-, 120-, and 80–70-kDa. Since it was shown that 3G6, but not 8F2, was co-stimulatory to a submitogenic dose of OKT3 mAb in peripheral T-cells, it is important to define the different signaling events associated with VLA-4-mediated T-cell co-stimulation. Therefore, we next identified each protein candidate in those bands by immunoprecipitation using mAbs, which recognize the known candidate proteins. We found that pp125FAK (focal adhesion kinase), paxillin, Fyn, and Lck were clearly tyrosine-phosphorylated in peripheral T-cells stimulated by solid-phase cross-linking using 3G6, but not using 8F2. This result indicated that the pp120 protein was pp125FAK, pp70 and pp50 proteins were paxillin, and pp60–55 proteins were p59fyn and p56lck. The 140-kDa protein was shown to be PLC-γ (phospholipase c-γ), and pp45 was tyrosine-phosphorylated MAP (mitogen-actirated protein) kinase (ERK1/2).[20] These results suggested that those tyrosine-phosphorylated proteins may play an important role in VLA-4-mediated T-cell co-stimulatory signaling events.

Identification of pp105 as a Cas-related protein

Although we identified several tyrosine-phosphorylated proteins stimulated by the ligation of VLA-β1 integrins in T-cells, a 105-kDa tyrosine-phosphorylated protein (pp105) had not yet been identified. pp105, a protein which we first identified in T-lymphoblastoid H9 cells as well as in peripheral T-cells, is tyrosine-phosphorylated by the engagement of VLA-4. In a previous study,[21] we demonstrated that pp105 and pp125FAK were tyrosine-phosphorylated by VLA-β1 integrin stimulation in similar kinetics in H9 cells, although pp105 is a distinct molecule from pp125FAK. Because pp125FAK is an essential tyrosine kinase for VLA-β1-integrin-mediated protein tyrosine phosphorylation, we attempted to define the relationship between pp125FAK and pp105. For this purpose, H9 cells were incubated with FN- or PLL (poly-L-lysine)-coated plates before cell lysis, subjected to precipitation with GST (glutathione S-transferase) fusion protein of the pp125FAK COOH-terminal domain (CT) (residues 706–1052, designated GST-CT), and analyzed by immunoblotting with anti-phosphotyrosine mAb (anti-pTyr). We observed that a tyrosine-phosphorylated 105-kDa protein was co-precipitated with GST-CT from FN-stimulated cell lysate, which migrated at the same position as pp105 and was detected only minimally in PLL-incubated cell lysate.[22] Paxillin was also co-precipitated with GST-CT from H9 cell lysate and was detected by anti-pTyr as a 70-kDa band, as we reported previously.[23] To determine whether pp105 binds to FAK or paxillin, H9 cell lysates were precipitated with deletion mutants of GST-CT. It was shown that pp105 was precipitated with GST–FAK residues 706–904, but not with GST–FAK residues 896–1052. Conversely, paxillin was precipitated with GST–FAK residues 896–1052, but not with GST–FAK residues 706–904. These results demonstrate that the pp105-binding domain of FAK is distinct from the paxillin-binding domain of FAK, indicating that pp105/FAK binding is not mediated by paxillin (Fig. 1). In contrast, a 130-kDa tyrosine-phosphorylated protein was precipitated with the GST–FAK fusion protein from human breast-cancer-derived T-47D cell lysates. Since it was reported that p130 Cas (Crk-associated substrate) bound to FAK by its SH3 domain,[24] we identified this 130-kDa protein as p130Cas using anti-Cas mAb. FAK residues 706–

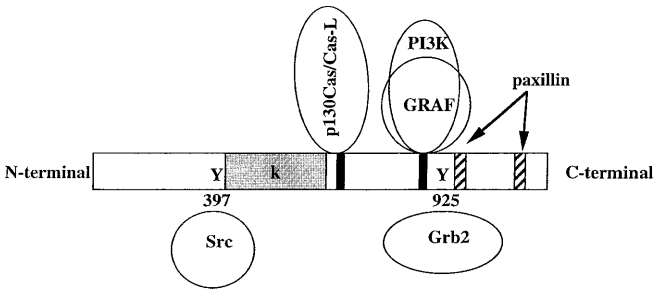

Fig. 1. Structure of pp125FAK (focal adhesion kinase). *K*, tyrosine kinase domain; *shaded boxes*, FAT (focal adhesion targeting) domain; *black boxes*, proline-rich sites, binding sites for Cas, PI-3K and GRAF. 397Y represents the binding site of the Src SH2 domain, and 925Y that of the Grb2 SH2 domain

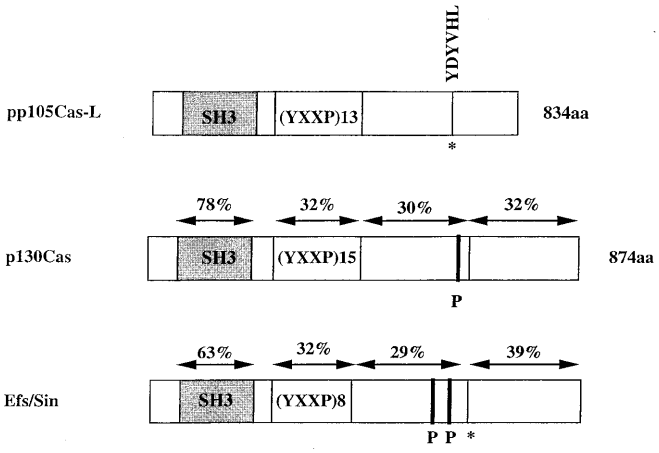

Fig. 2. Structure of pp105Cas-L and Cas-family proteins. Comparison of amino acid sequences among Cas proteins. Amino acid sequence homologies between Cas-L and the other Cas protein in the SH3 domain, substrate domain (Cas-L residues 92–348), specific domain (Cas-L residues 349–628), and CT (Cas-L residues 629–834) are shown above each domain. YDYVHL motifs are shown by *asterisks* and vertical lines. Proline-rich sequences are marked *P* with heavier vertical lines

904, which were sufficient for pp105 binding, contained the reported p130Cas-binding site. Based on this information, we demonstrated that pp105, precipitated with GST-CT from the lysate of H9 cells, was reactive with anti-Cas mAb, and that the 105-kDa protein, precipitated with anti-Cas mAb, showed the same migration on SDS–PAGE as pp105, which was precipitated by the GST-CT. Further sequential precipitation and immunodepletion analyses have confirmed that pp105, a 105-kDa protein that is tyrosine-phosphorylated by β1-integrin stimulation, is a Cas-related protein.

cDNA cloning of pp105

To further determine the structure of pp105, we screened the λgt11 cDNA library derived from a human T-lymphoblastoid cell line (Hut78) with anti-Cas mAb. Nucleotide sequences of three independent clones had homology with p130 Cas.[20] These three clones were cDNAs of an identical transcript, and the nucleotide sequences contained an open reading frame of 834 amino acids. The deduced amino acid sequences of this transcript showed conserved motifs with p130 Cas, one SH3 (Src homology 3) domain in the NH2-terminal region, and multiple putative binding sites for the SH2 domains (Fig. 2). Most of the SH2 binding motifs in the substrate domain are YXXP (YDXP), which are putative binding sites for Crk, Nck, and Abl SH2 domains.[25] Despite the conserved motifs, homology between p130Cas and the deduced amino acid sequence of this cDNA is relatively low (78% in the SH3 domain, 32% in the substrate domain, 30% in the specific domain, and 32% in the CT). Homology with another Cas-related protein, Efs/Sin[26] is also relatively low. These results indicate that cDNA encodes a novel Cas-related protein. p130Cas was highly phosphorylated on tyrosine residues in v-Src- or v-Crk-transformed cells.[27] In a similar way to p130Cas, pp105 was also highly phosphorylated on tyrosine residues by the co-transfection of Src, Lck, CrkI, or CrkII. Moreover, tyrosine-phosphorylated pp105 binds to Crk proteins in vivo. Since pp105 is preferentially expressed in lymphocyte, we designated pp105 as Cas-L (Cas-lymphocyte type).

We next attempted to define the proteins recruited to Cas-L in a phosphorylated tyrosine residue-dependent manner. For this purpose, lysates from FN-stimulated H9 cells were precipitated with GST fusion proteins containing SH2 domains from various proteins and analyzed by immunoblotting with anti-Cas mAb and anti-pTyr. As a result, Cas-L was precipitated with GST–SH2-domain fusion proteins of c-Abl, Crk, and Nck, and weakly precipitated with those of Lck, SHPTP2, and Csk.[22] The binding of Cas-L and those GST–SH2 fusion proteins were shown to be enhanced upon the engagement of β1 integrin, indicating that tyrosine-phosphorylated Cas-L binds to SH2 domains of c-Abl, Crk, Lck, Nck, Csk, and SHPTP2 in vitro. Furthermore, in vivo co-precipitation analysis showed that Cas-L was co-precipitated with Crk and Nck, and weakly co-precipitated with SHPTP2. Unlike pp125FAK–Cas-L binding, the amount of Cas-L co-precipitated with Crk, Nck, or SHPTP2 was increased by VLA-β1 integrin stimulation with FN. These results indicate that VLA-β1 integrin stimulation leads to the recruitment of Crk, Nck, and SHPTP2 to the tyrosine-phosphorylated Cas-L, in addition to stimulation-independent association with pp125FAK. These protein–protein interactions further suggest the putative functions of Cas-L in the VLA-β1 integrin-mediated signaling pathways.

Tyrosine phosphorylation of Cas-L and its recruitment of Crk and C3G upon CD3 crosslinking

Recently, we reported that the conserved YDYVHL sequence of Cas-L was phosphorylated by FAK following β1 integrin crosslinking, and that an Src-family tyrosine

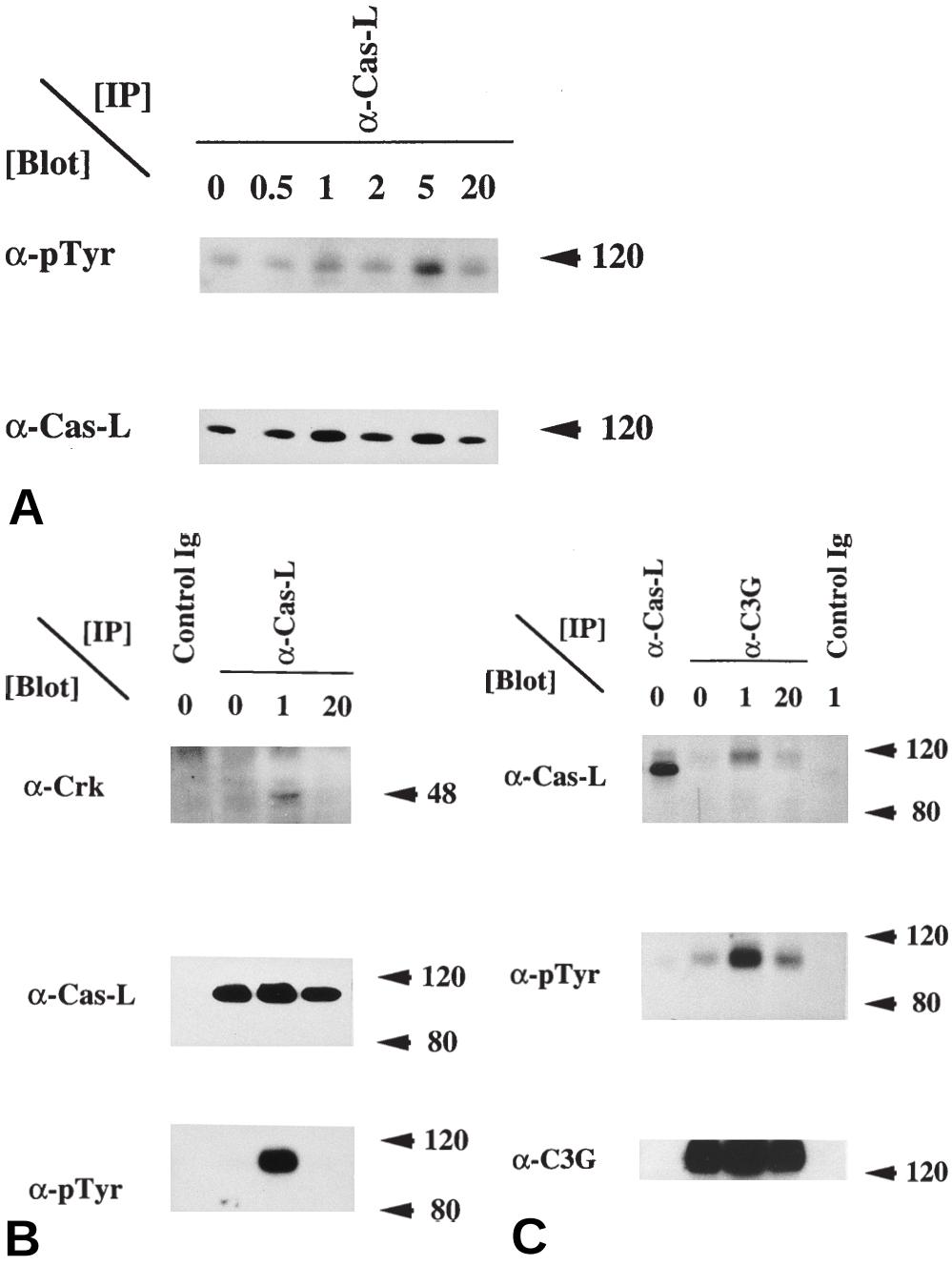

Fig. 3. **A** Cas-L is tyrosine-phosphorylated after the CD3 crosslinking in T-cells. Peripheral T-lymophocytes stimulated with OKT3 for the times indicated were lysed and then immunoprecipitated with anti-Cas-L Ab. The immunoprecipitates were analyzed by Western blotting with the same antibody, followed by ^{125}I-labeled anti-pTyr. **B** Cas-L binds to Crk in a phosphorylation-dependent manner. H9 cells were stimulated with OKT3 for the times indicated. Cell lysates were immunoprecipitated with control or anti-Cas-L Ab. The immunoprecipitates were blotted with anti-Crk mAb and anti-Cas-L Ab, followed by ^{125}I-labeled anti-pTyr. **C** Cas-L forms complexes with C3G following CD3-stimulation in T-cells. Immunoprecipitates from CD3-stimulated H9 cell lysates using anti-Cas-L, anti-C3G, or control Ab were analyzed by Western blotting with anti-Cas-L or anti-C3G Ab, followed by ^{125}I-labeled anti-pTyr

kinase(s) is recruited to the phosphorylated YDYVHL sequence, which causes further tyrosine phosphorylation of Cas-L.[28] Although the significance of Cas-L in β1 integrin-mediated signaling has been well documented, little is known about its ability in other signaling pathways. Since Cas-L is predominantly expressed in lymphocytes,[22] it is conceivable that Cas-L may participate in lymphocyte-specific signaling pathways. First, we attempted to determine whether Cas-L is tyrosine-phosphorylated upon CD3 crosslinking.[29] The CD3 molecule on the normal peripheral T-lymphocytes and H9 cells was crosslinked with anti-CD3 mAb. As shown in Fig. 3A, Cas-L was tyrosine-phosphorylated 1 min and 5 min after CD3 crosslinking, and then dephosphorylated 20 min later, showing similar kinetics to the case of p59Fyn and p56Lck (data not shown). While both FAK and Cas-L were tyrosine-phosphorylated following β1 integrin crosslinking, FAK was not significantly phosphorylated upon CD3 crosslinking. These results strongly suggest that Cas-L is involved in the TCR/CD3 signaling pathway which is independent of FAK. We next defined binding molecules to tyrosine-phosphorylated Cas-L. For this purpose, H9 cells were lysed after CD3 crosslinking, and were then subjected to immunoprecipitation with anti-Cas-L mAb, and analyzed by immunoblotting with anti-Crk-specific mAb and anti-pTyr mAb. As shown in Fig. 3B, Crk was co-precipitated with Cas-L 1 min after CD3 crosslinking, but disappeared 20 min after CD3 stimulation. We also found that Cas-L was precipitated by the GST-Crk

SH2 domain (data not shown). Taken together, these results indicate that Cas-L associates with Crk through the Crk SH2 domain following CD3 crosslinking in a tyrosine-phosphorylation-dependent manner. One of the major Crk-binding proteins via the Crk SH3 domain is C3G, a guanine exchange factor for Rap1A/K-rev1.[30,31] Therefore, we next investigated the CD3-dependent Cas-L-C3G interaction. As shown in Fig. 3C, Cas-L was coprecipitated with C3G 1 min following CD3 crosslinking. These results suggest that tyrosine-phosphorylated Cas-L binds to C3G possibly through the association with Crk upon CD3 crosslinking in T-cells.

Involvement of Cas-L in β1 integrin-mediated T-cells co-stimulation and migration

Despite the significance of these biochemical findings, the biological functions of Cas-L in the β1 integrin-mediated signal transduction during immune response have not been characterized. It is well known that the binding of T-cells to ECM through β1 integrins provides co-stimulatory signals to CD3-dependent T-cell proliferation and IL-2 production.[5–9,19,32,33] Since it has been shown that Cas-L is the substrate for FAK and is tyrosine-phosphorylated by β1 integrin stimulation, we analyzed the roles of Cas-L in β1 integrin-mediated co-stimulation using the human T-lymphoblastoid cell line, Jurkat.[34]

As is well established, stimulation with the co-immobilized anti-CD3 mAb and anti-CD29 mAb, anti-CD49d mAb, or the GST–CS1 fusion protein, or anti-CD28 mAb induces IL-2 production in human peripheral-T-cells. In the absence of anti-CD3 mAb, IL-2 production was not induced by any of these stimuli. In contrast, the β1 integrin-mediated signaling pathway is selectively impaired in Jurkat cells. As shown in Fig. 4A, ligation of β1 integrin by mAbs or CS1 co-immobilized with anti-CD3 mAb did not induce IL-2 production in Jurkat cells, whereas co-crosslinking of CD28 and CD3 caused substantial IL-2 production in the same cells. Since we observed the normal expression of VLA-4 on the surface of Jurkat cells, we presumed that the intracellular signaling pathway from β1 integrin was impaired in Jurkat cells, and found that the expression of Cas-L in Jurkat cells was marginal.[23] This finding led us to hypothesize that the lack of β1 integrin-mediated co-stimulation in Jurkat cells is due to insufficient expression of Cas-L. We next developed stable transformants of Jurkat cells that express wild-type or SH3-deleted mutant Cas-L (Cas-LΔSH3). Surprisingly, as shown in the same figure, stimulation by the co-immobilized CS1 and anti-CD3 mAb induced a substantial IL-2 production in the wild-type Cas-L transfected Jurkat cells, but not in the vector control. This increased IL-2 production in Jurkat–Cas–Lwt cells was inhibited by the addition of either anti-CD49d or anti-CD29 mAb. In concert with that, anti-CD49d mAb, 3G6[20] also provided a dose-dependent co-stimulation in those cells. Next, we analyzed Jurkat transfectant of a Cas-LΔSH3 mutant which lacks the SH3 domain and does not bind to

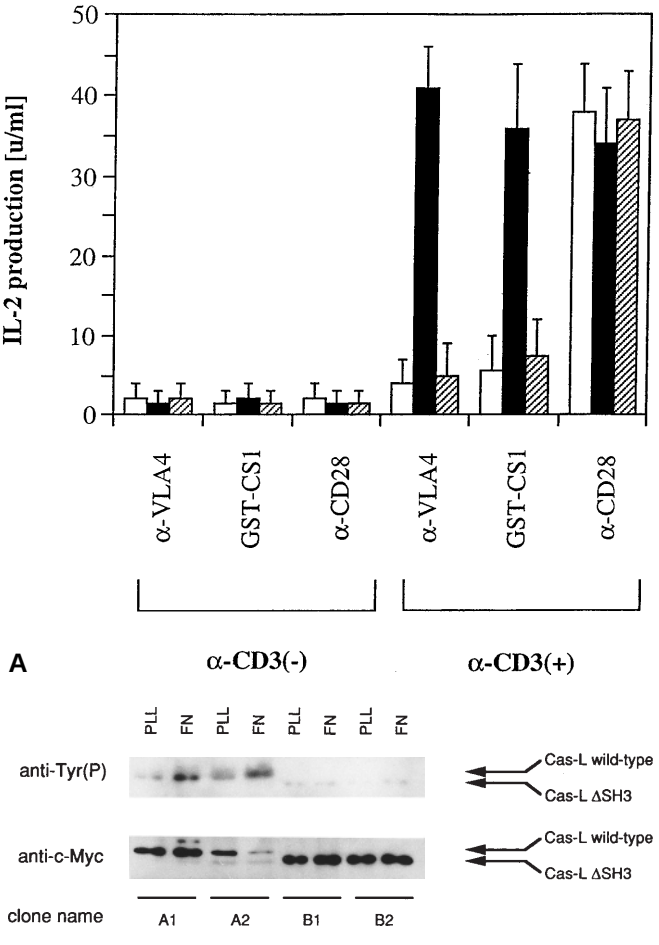

Fig. 4. The SH3 domain of Cas-L is essential for β1 integrin-mediated co-stimulation of IL-2 production and Cas-L phosphorylation. **A** Absence of β1 integrin-mediated co-stimulation of IL-2 production in vector control and Cas-LDSH3 transfected Jurkat cells. Jurkat cell clones that were transfected with Cas-L wild type (*solid bars*), Cas-LΔSH3 (*shaded bars*), or vector (*open bars*) were cultured for 24 h on plates coated with anti-CD3 mAb (0.5 μg/ml) plus anti-CD49d (10 μg/ml), GST-CS1 fusion protein (10 μg/ml), or anti-CD28 (10 μg/ml). The secretion of IL-2 was measured by CTLL-2 assay. **B** FN-mediated tyrosine phosphorylation of Cas-L in Jurkat transfectants. Jurkat transfectants (A1, A2: Cas-L wild type; B1, B2: Cas-LΔSH3 mutant) were stimulated with poly-L-lysine (PLL) control or FN for 1 h and lysed on plates using 1% NP-40 lysis buffer. Lysates were immunoprecipitated with anti-c-myc epitope antibody (9E10). Immunoprecipitates were separated by SDS-PAGE, and blotted with 9E10 and anti-phosphotyrosine mAb

pp125FAK.[28,35] As shown in Fig. 4A, stimulation of Jurkat-ΔSH3 cells with immobilized anti-CD3 mAb plus either anti-CD49d mAb or CS1 failed to induce significant levels of IL-2 production.

To define the molecular basis of the difference in IL-2 production between wild-type Cas-L and Cas-LΔSH3, we examined tyrosine phosphorylation of these Cas-L proteins following stimulation with immobilized CS1. As shown in Fig. 4B, adhesion of the transfectants to FN- or CS1-coated plates induced tyrosine phosphorylation of wild-type Cas-L, but not Cas-LΔSH3. This finding indicates that the SH3

domain of Cas-L is required for its tyrosine phosphorylation upon engagement of β1 integrin. It is thus suggested that the binding of Cas-L to pp125FAK is crucial for β1 integrin-mediated tyrosine phosphorylation of Cas-L, and that tyrosine phosphorylation of Cas-L is critical in β1 integrin-mediated co-stimulation of IL-2 production.

Since β1 integrin appears to be involved in T-cell migration, we also studied the involvement of Cas-L in CD3-induced T-cell migration on fibronection (FN) using Boyden chamber assays.[36] The migratory response was induced in the Cas-L-transfected Jurkat cells following the ligation of CD3 and β1 integrin, whereas parent Jurkat cells that marginally expressed Cas-L did not migrate in the same condition. Furthermore, we note that FN alone provides migration signals significantly in these Cas-L transfected cells, although the level of migration is lower than that of CD3 plus FN stimulation. The ligation of CD3 and β1 integrin induces Cas-L tyrosine phosphorylation, which is associated with migratory behavior. Taken together with the above findings, it is suggested that Cas-L is a key molecule in T-cell co-stimulation and migration induced by the ligation of CD3 and β1 integrin.

Concluding remarks

The study outlined in this article was initiated to elucidate the mechanism of the co-stimulatory nature of integrin engagement to TCR-mediated cell signaling in T-lymphocytes.[5,7,24] TCR-antigen binding induces gene expression, cytokine production, and cell proliferation in T-lymphocytes.[37] These TCR-dependent signals are mediated by tyrosine phosphorylation of various proteins, including CD3δ, CD3ε, CD3γ, ZAP-70, Shc, Vav, and c-Cbl.[35] These signaling molecules appear to be involved in the phosphorylated tyrosine-mediated protein–protein interaction and the recruitment of the other signaling molecules containing Src homology (SH)2 domains.[25] The recruitment of these signaling molecules is essential to induce various signals to the downstream events such as the activation of mitogen-activated protein kinases, Ca^{2+} influx, and transcriptional activation of various genes.[37,38] Thus, protein tyrosine phosphorylation plays a key role during the initial phase of TCR-mediated T-cell activation. In pursuit of the molecular mechanism of the β1 integrin-mediated co-stimulatory signal, we found that a distinct set of proteins was phosphorylated in their tyrosine residues upon the engagement of VLA-β1 integrin.[20] Among those proteins, PLC-γ, p59fyn, p56lck, and ERK1/2 are supposed to participate in TCR-mediated signaling pathways. Integrin-mediated tyrosine phosphorylation of those proteins may result in the augmentation or sustenance of the TCR-mediated signal. It is thus suggested that these findings may provide an important clue to one of our major questions regarding the crosstalk between TCR- and integrin-mediated signaling pathways. Furthermore, we have successfully identified pp105, a new molecule which is also tyrosine phosphorylated upon crosslinking of β1 integrins. The pp105 protein has been shown to belong to a Cas family, and consists of p130Cas, Cas-L/HEF-1/pp105, and Efs/Sin, which are tyrosine-phosphorylated upon various stimuli including integrin-mediated cell adhesion, growth factors, chemokines, and crosslinking of the B-cell receptor.[19,22,39–42] The three proteins share the common structural characteristics of an N-terminal SH3 domain, followed by multiple (8–15) YXXP motifs which are putative binding sites for the Crk SH2 domain, and a conserved YDYVHL sequence that possibly binds to the Src SH2 domain.[22,26,27] Tyrosine-phosphorylated Cas family proteins bind to signaling molecules containing SH2 domains.[22,43,44] Of particularly interest is that Cas-L is preferentially expressed in lymphocytes, which led us to investigate its roles in TCR- and β1-integrin-mediated cell signaling in T-cells.

We demonstrated a requirement of Cas-L for the β1 integrin-mediated co-stimulatory signaling in Jurkat T-cells. Furthermore, we suggested that tyrosine phosphorylation of Cas-L is necessary for the β1 integrin-mediated co-stimulation in Jurkat cells. How does tyrosine-phosphorylated Cas-L transfer signals in these cells? Since the tyrosine-phosphorylated form of Cas-L recruits Crk, Nck, and SHP2 in an SH2 domain-dependent manner,[20] these molecules may transduce signals downstream from Cas-L following β1 integrin stimulation in T-cells.

We have demonstrated that Cas-L is tyrosine-phosphorylated following CD3 crosslinking as well as the engagement of β1 integrin, which subsequently forms complexes with Crk and C3G. These results demonstrated a novel signaling pathway through Cas-L following TCR stimulation. The other question to be solved concerns the missing link between the binding of Crk to Cas-L and the transcriptional regulation of various genes which occurs on T-cell activation. Crk is an adapter protein composed of one SH2 domain and one or two SH3 domains, and binds to various proteins including C3G, Dock180, Sos, and c-Abl via the Crk SH3 domain.[31,45–47] This suggests that Cas-L can recruit these signaling molecules in a tyrosine phosphorylation-dependent manner via Crk. C3G is a guanine nucleotide exchange factor that activates a small GTPase, Rap1A/K-rev1,[31] which was first reported to revert the K-Ras transformed phenotype of NIH3T3 cells.[46] Rap1A has been shown to inhibit the binding of Ras to Raf-1 and Ras-dependent Raf-1 activation.[49] Therefore, the recruitment of C3G to Cas-L may be involved in the regulation of ras-mediated signaling pathways. The other putative signal downstream of C3G is the JNK/SAPK pathway. Tanaka et al.[50] recently reported that over-expression of Crk induces activation of JNK/SAPK, and that co-expression of C3G further enhances the JNK activity. Over-expression of Crk has been shown to induce tyrosine phosphorylation of Cas-L and p130Cas, as well as the recruitment of C3G to the phosphorylated Cas proteins.[22,27] On the other hand, it has been reported that JNK is activated upon TCR stimulation, and that JNK activation plays a significant role in T-cell activation.[51] These findings suggest that Cas-L is involved in TCR-mediated JNK activation through the recruitment of C3G. In addition, Nck and SHP2 were also demonstrated to be involved in the activation of ERK.[42,52] Therefore, it is

possible that the recruited Crk, Nck, and/or SHP2 induce expression and activation of transcription factors through regulation of ERK upon the engagement of β1 integrins. Since Ras/Raf-1/ERK and JNK pathways have been shown to serve an important function in T-cell activation,[51,53] Cas-L may regulate T-cell responses through these pathways upon engagement of TCR and/or β1 integrins.

Another question is the mechanism by which Cas-L phosphorylated upon CD3 crosslinking. We reported that activated p56lck phosphorylates Cas-L,[22] and we also found that the co-expression of ZAP-70 also phosphorylates Cas-L (unpublished observation), whereas FAK phosphorylates Cas-L upon the stimulation through β1 integrins.[22] As both p56lck and ZAP-70 are involved in TCR/CD3 signaling pathways,[37,38] these tyrosine kinases are likely involved in the CD3-dependent phosphorylation of Cas-L.

In summary, pp105/Cas-L is a unique docking protein which may serve as a site of convergence of signals through β1 integrins and TCR, as well as a variety of phosphotyrosine-mediated intracellular signaling molecules. The approaches outlined in this article will shed light on the basic understanding of lymphocyte activation and motile behavior, as well as the pathophysiological mechanisms of rheumatic disorders.

Finally, recent efforts in the field of β integrins will provide some insights into the immunopathogenesis and therapeutic intervention of a variety of rheumatic disorders, including rheumatoid arthritis and systemic lupus erythematosus (SLE).

The area of β1 integrin molecules and their signaling pathway promises to remain a fruitful area of research and should continue to provide critical clues as to the development of a novel therapy for rheumatic disorders as well as other inflammatory diseases.

Acknowledgments This work was supported in part by grants from the Ministry of Education, Science, Sports, and Culture, and the Ministry of Health Welfare of the Japanese government, and by NIH Grants AR-33713 and AI-29530. We thank A. Wakamatsu for indispensable secretarial assistance.

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