T Cell Activation through the CD43 Molecule Leads to Vav Tyrosine Phosphorylation and Mitogen-activated Protein Kinase Pathway Activation

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CD43, the most abundant membrane protein of T lymphocytes, is able to initiate signals that lead to Ca\(^{2+}\) mobilization and interleukin-2 production, yet the molecular events involved in signal transduction pathway of the CD43 molecule are only beginning to be understood. We have shown recently that cross-linking CD43 on the cell surface of human T lymphocytes with the anti-CD43 monoclonal antibody L10 leads to CD43-Fyn kinase interactions and to Fyn phosphorylation on tyrosine residues. This interaction seems to be mediated by the SH3 domain of Fyn and a proline-rich sequence located in the cytoplasmic domain of CD43. Here we show that CD43-specific activation of human T lymphocytes induced tyrosine phosphorylation of the adaptor protein Shc and of the guanine exchange factor Vav, as well as the formation of a macromolecular complex that comprises Shc, GRB2, and Vav. CD43 ligation resulted in enhanced formation of Vav-GRB2-SLP-76 complexes and in the activation and nuclear translocation of ERK2. Cross-linking of the CD43 molecule in 3T3-CD43 \(^{+}\) cells induced luciferase activity from a construct under the control of the Fos serum responsive element. Altogether, these data suggest that the mitogen-activated protein kinase pathway is involved in CD43-dependent interleukin-2 gene expression.

T cell-mediated immune responses are triggered when the antigen-specific T cell receptor (TcR)\(^z\) interacts with antigen in the context of the major histocompatibility complex molecules, leading to a cascade of signaling events that culminate with the activation of T lymphocytes. Modulation of T cell signaling is contributed by the concerted actions of co-receptor molecules such as CD2, CD4, CD8, and CD28. It has been reported that the signals generated by these cell-surface molecules activate specific tyrosine kinases involved in T cell signaling such as Lck, Fyn, and Zap-70. Autophosphorylation of these kinases will provide tyrosine-phosphorylated motifs for SH2-mediated protein-protein interactions. Among those targets, adaptor proteins such as Shc (1) and Cbl (2–4) become phosphorylated on tyrosine residues after T cell activation. Phosphorylation of these adaptor proteins favors protein-protein interactions that lead to the formation of macromolecular complexes involving effector proteins such as the guanine nucleotide exchange factors (GEFs) Sos (5) and Vav (6). Shc-GRB2-Sos complexes formed following TcR engagement leads to Rac activation (7, 8), a critical checkpoint for cell proliferation and gene expression. On the other hand, a role for Vav in T cell activation has been pointed out by data showing that Vav activates Rac 1 (9, 10) and that the association of Vav to the adaptor protein SLP-76 participates in TcR-dependent IL-2 gene expression (11, 12).

The T cell-surface glycoprotein CD43, also called sialophorin, gp115, or leukosialin, is a transmembrane protein consisting of three domains as follows: a highly O-glycosylated extracellular domain of 235 amino acids; a transmembrane domain of 23 amino acids; and an intracytoplasmic domain that is 123 amino acids long (13, 14). Post-translational modifications of the glycosylation pattern of the protein backbone result in two isoforms of CD43: a 115-kDa protein as well as a 130-kDa one. The 115-kDa form of CD43 is on all T cells, whereas the 130-kDa is more abundant on resting CD8\(^{+}\) cells and is up-regulated both on CD4\(^{+}\) and CD8\(^{+}\) lymphocytes upon activation (15–17). Three natural ligands have been described for CD43, ICAM-1 (18), galecin-1 (19), and more recently, major histocompatibility complex I (20), suggesting a complex role for this protein in regulating cellular adhesion and hence cellular activation through the specific interaction of each isoform with its ligand(s).

Although CD43 is the most abundant T cell-surface glycoprotein, its functions are not yet well defined. The participation of CD43 in cellular adhesion remains controversial. Some reports provide evidence that CD43 regulates the interaction of integrins with their ligands by an unknown mechanism (21). A possible role for CD43 in T cell homing has been pointed out by the fact that one particular anti-CD43 mAb inhibits T cell homing (22). On the other hand, the negative charge of the sialic acid residues of CD43 has been suggested to contribute to anti-adhesive processes (23, 24). The interaction of CD43 on immature cortical thymocytes with galecintin-1 on thymic epithelial cells may play a role in the positive selection of thymocytes (25, 26). The role of CD43 as a receptor molecule involved in cellular activation through an independent signal transduction pathway has been suggested. CD43 ligation with different monoclonal antibodies (mAbs) induced the generation of diacylglycerol and inositol phosphates, Ca\(^{2+}\) mobilization, and protein kinase C activation (27). These effects are mediated by the highly conserved cytoplasmic domain of CD43 since deletion of the entire cytoplasmic domain abolished the co-receptor functions of CD43 when expressed in a murine T cell hybridoma (28).
The molecular events involved in the CD43-dependent signaling pathway have only begin to be elucidated. We have recently shown that cross-linking CD43 on the cell surface of human T lymphocytes with the anti-CD43 mAb L10 leads to CD43-Fyn kinase interaction and to Fyn phosphorylation on tyrosine residues. This interaction is mediated by the Fyn SH3 domain and presumably a proline-rich sequence located in the cytoplasmic domain of CD43 (29). Here we report that CD43-specific activation of human T lymphocytes induces tyrosine phosphorylation of the adaptor protein Shc and of the guanine exchange factor Vav as well as the formation of a macromolecular complex that comprises Shc, GRB2, and Vav. We also show that CD43 ligation enhances the formation of Vav-SLP-76 complexes and that CD43 signaling from the cell surface to the nucleus involves the MAP kinase (MAPK) pathway.

MATERIALS AND METHODS

Reagents—L10, an IgG1 mAb that recognizes CD43 (Remold-O'Donnell et al. (66)), was either purified from ascites on protein A-Sepharose columns or used as ascites (1:500). Rabbit anti-mouse IgG antiserum (RmMIG) was generated by repeated immunizations with purified mouse IgG, and anti-mouse IgG immunoglobulins were affinity purified. S6E, an IgG1 mAb that recognizes the Vp7 protein from human rotavirus (30), was used as a negative control in all experiments and was the kind gift from Dr. Luís Paśiñor-Noriega, Instituto de Biocinética/UNAM. The anti-GRB2, anti-Vav, and anti-ERK2 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-SLP-76 antibody was kindly provided by Dr. Koreszky, University of Iowa. The anti-phosphotyrosine 4G10 mAb was generously provided by Dr. Roberts, Dana Farber Cancer Institute. The SRE-Luc plasmid was a gift from Dr. Kaibuchi, Nara Institute of Science and Technology.

Human T Cells—Peripheral blood T cells were isolated from healthy adult donors by Ficoll-Paque gradient centrifugation. Briefly, the buffy coat was washed three times with phosphate-buffered saline and resuspended antibodies (RPMI) supplemented with 5% fetal calf serum (FCS) (Hyclone) and 5% bovine iron-supplemented calf serum (Hyclone), 2 mM L-glutamine (Sigma), 50 units/ml penicillin, 50 units/ml streptomycin, and 50 mM β-mercaptoethanol. Adherent cells were removed by pelting the cells onto 10-mm Petri dishes (4 × 10^6 cells/plate) for at least 2 h at 37 °C in a 5% CO2 atmosphere. Non-adherent cells were collected and loaded on a nylon wool column pre-equilibrated with cold RPMI. The resultant purified cells were predominantly (>80%) OKT3+ and L10+ (>95%) as determined by fluorescence-activated cell sorter analysis, as described previously (29). Prior to stimulation, purified T cells were cultured for 24 h in RPMI-2% FCS.

Activation of T Cells and Immunoprecipitation—Purified T cells (2 × 10^6) were incubated in 0.5 ml of cold RPMI for 5 min at 4 °C with the following antibodies: L10 or S6E (1:500 dilution of ascites or 1:1000 dilution of purified IgG). Unless otherwise specified, cross-linking was achieved by further incubating the cells with RmMIG (1 μg/ml) for 15 min at 4 °C. Cells were activated by incubating them at 37 °C for different times as indicated in the text. Since no particular effect was detected after incubating the cells with the control mAb S6E for up to 20 min, in some experiments cells were treated with 3D6 for only 5 min. Cells were then lysed in 100 μl of lysis buffer (25 mM HEPES at pH 7.7, 150 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5% Triton X-100, 0.5 mM dithiorthreitol, 20 mM β-glycerophosphate, 1 mM Na3VO4, 5 mM NaF, 4 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin) for 30 min at 4 °C. Lysates were spun at 14,000 × g for 15 min at 4 °C, and supernatants were pre-cleared with protein A-Sepharose for 2 h at 4 °C. Lysates from activated or non-activated T cells (1 × 10^7 cellular equivalents) were then immunoprecipitated with the indicated antibody (1 μg/ml) for 1 h at 4 °C. Immune complexes were harvested with protein A-Sepharose for 30 min on ice and washed once with cold TNE-T (150 mM NaCl, 50 mM Tris, pH 7.5, 5 mM EDTA, 1% (v/v) Nonidet P-40), twice with TNE (150 mM NaCl, 50 mM Tris, pH 7.5, 5 mM EDTA), once with H2O, and finally washed with kinase buffer (20 mM Tris, pH 7.5, and 5 mM MgCl2). Beads were then incubated at 30 °C for 15 min with 100 μl of the same kinase buffer containing 10 μCi of [γ-32P]ATP (3000 Ci/mmol, NEN Life Science Products) and 5 μg of casein (Sigma) as exogenous substrate. The beads were spun down, and the supernatant was mixed with an equal volume of 2% loading buffer. Proteins were separated by SDS-PAGE, and radiolabeled proteins were visualized by x-ray film exposure. Levels of protein phosphorylation were quantitated with a densitometer, and results were expressed as fold induction as compared with unstimulated cells.

Nuclear Extracts—Stimulated T cells were incubated for 5 min at 4 °C in hypotonic buffer (10 mM Tris, pH 8.0, 10 mM KCl, 0.5 mM EDTA, 1 mM dithiothreitol, 1 μg/ml leupeptin, and 0.5 mM phenylmethylsulfonyl fluoride) prior to being lysed by vortexing. Nuclei were collected in the pellet by centrifugation at 2000 × g for 5 min at 4 °C; the supernatant (cytoplasmic fraction) was kept at −20 °C. Nuclei were washed twice in the same buffer, and nuclear proteins were extracted by incubation for 30 min at 4 °C in a buffer containing 20 mM Tris, pH 8.0, 450 mM KCl, 0.5 mM EDTA, 1 mM dithiothreitol, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 5 mM spermidine, and 25% glycerol. Extracts were clarified by centrifugation at 14,000 × g for 10 min at 4 °C. The protein concentration of the nuclear extracts and the cytoplasmic fraction was determined by the Bradford method, and an equal amount of proteins was separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with anti-Erk2 antibodies.

RESULTS

CD43-dependent T Cell Activation Induces Shc Phosphorylation and the Interaction of Shc with GRB2—We have previously shown that cross-linking CD43 on the T cell surface leads to the interaction of CD43 with Fyn and to Fyn phosphorylation on tyrosine residues. In order to identify other molecules involved in the CD43 signaling pathway, lysates from resting or CD43-activated human T cells were analyzed by immunoblotting with the anti-phosphotyrosine antibody 4G10. Cross-linking CD43 on the cell surface with the L10 mAb induced the phosphorylation of several proteins, including proteins of approximately 52 and 95 kDa.2

The adaptor protein Shc has been shown to participate in T cell receptor signaling pathways (1). Three isoforms, generated from the same gene by translational frameshift (p47, p52, and p66), have been described for Shc (32, 33). In order to investigate whether the 52-kDa phosphotyrosine protein observed in total cell extracts from CD43-activated T lymphocytes was Shc,
we immunoprecipitated Shc from precleared cell lysates and looked for its phosphorylation state by immunoblotting with anti-phosphotyrosine antibodies. As shown in Fig. 1A (top panel), Shc immunoprecipitates contained a 52-kDa protein whose tyrosine phosphorylation level increased with time after CD43 cross-linking (lanes 1–5), whereas no tyrosine phosphorylation of the 52-kDa protein was observed in Shc immunoprecipitates from T cells treated with the control antibody 3D6 (lane 6–9). Probing the same membrane with anti-Shc antibodies revealed a band at the expected molecular weight (Fig. 1A, lower panel), thus indicating that Shc is tyrosine-phosphorylated following CD43 ligation on T cells. Surprisingly, Shc immunoprecipitates contained also a 95-kDa protein and the degree of phosphorylation of this protein also increased with time after CD43 cross-linking (Fig. 1A, top panel, lanes 1–5). The phosphorylation of p95 was specific for CD43 signaling since p95 phosphorylation levels did not increase when Shc was immunoprecipitated from T lymphocytes treated with the irrelevant mAb 3D6 (Fig. 1A, lanes 6–9). These data indicate that p52 is the adaptor protein Shc, that it is tyrosine-phosphorylated, and associates with a protein of 95 kDa upon CD43 cross-linking in T lymphocytes. T cell activation mediated by several receptor molecules (34–36) has been shown to induce the interaction of Shc with the adaptor protein GRB2. We tested the possibility that Shc could interact with GRB2 following CD43 activation. Total cell lysates were immunoprecipitated with anti-GRB2 antibodies after T cell activation with the L10 mAb. As shown in Fig. 1B (top panel), Shc was co-immunoprecipitated with GRB2 following CD43 cross-linking for different times. Furthermore, the Shc molecules interacting with GRB2 were tyrosine-phosphorylated in response to CD43 activation. Probing the same membrane with anti-GRB2 antibodies showed the presence of GRB2 in all immunoprecipitates (Fig. 1B, lower panel). Together, these results provide evidence that CD43-specific activation of human peripheral T lymphocytes induces tyrosine phosphorylation of the adaptor protein Shc as well as its association with the adaptor protein GRB2 and a p95-kDa protein.

Vav Tyrosine Phosphorylation Is Induced upon CD43 Cross-linking—The formation of macromolecular complexes containing Shc, GRB2, and Sos leading to activation of the Ras signaling pathway has been reported in different cells (37–38). However in T lymphocytes, the 95-kDa protein Vav is also tyrosine-phosphorylated upon TcR cross-linking. Vav tyrosine phosphorylation parallels an increase in the GTPase activity of Rac 1, suggesting an important role of Vav in T cell signaling (10, 39). Since Vav can also interact with Shc and GRB2 (40, 41), we investigated whether the 95-kDa protein that is tyrosine-phosphorylated upon CD43 cross-linking could be Vav. Vav was immunoprecipitated from pre-cleared lysates from CD43-activated T lymphocytes. Fig. 2A (top panel) shows that CD43-mediated T cell activation induced Vav phosphorylation on tyrosine residues in a time-dependent manner. After 5 min activation, Vav phosphorylation was 4-fold higher when compared with the levels detected when cells were treated with the control antibody 3D6 for 5 min (compare lanes 4 and 6). As seen in Fig. 2A (lower panel), comparable amounts of Vav were immunoprecipitated in all lanes. Vav immunoprecipitates from the L10-activated lymphocytes also contained tyrosine-phosphorylated Shc (Fig. 2B, top panel); although Shc phosphorylation on tyrosine residues.
CD43-dependent T Cell Activation

**Fig. 3.** CD43 dimerization is sufficient for Vav and Shc phosphorylation. Purified T lymphocytes (2 × 10⁷) were activated with anti-CD43 L10 mAb and RaMIG (lanes 1–3) or with L10 mAb alone (lanes 4 and 5) for the indicated times. Pre-cleared lysates were immunoprecipitated (IP) with anti-Vav antibodies. After transferring to nitrocellulose, proteins were blotted with anti-phosphotyrosine (Anti-PY) 4G10 mAb. Data shown are representative of two independent experiments.

**Fig. 4.** CD43 ligation enhances SLP-76-Vav interaction. Purified T cells (2 × 10⁷) were activated by CD43 cross-linking with L10 mAb (lanes 1–5) or with the isotype control 3D6 mAb (lanes 6–9) for the indicated times. SLP-76 (A) or Vav (B) were immunoprecipitated (IP) from pre-cleared lysates; immune complexes were resolved by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with 4G10 mAb (A, top panel) or anti-Vav antibodies (A and B, bottom panel) or with anti-SLP-76 antibodies (B, top panel). This figure corresponds to one out of four identical experiments. Anti-PY, anti-phosphotyrosine.

**CD43 Dimerization Is Sufficient for Shc and Vav Phosphorylation—**To determine whether maximum cross-linking of CD43 was necessary to initiate a signaling cascade, T lymphocytes were activated at 37 °C for different times following CD43 cross-linking with either the mAb L10 alone or L10 and RaMIG. Ligation of CD43 with L10 alone resulted in equivalent levels of Shc phosphorylation after 5 min activation, as compared with those observed after cross-linking with L10 and RaMIG (Fig. 3, lanes 3 and 5). However, the kinetics of Vav phosphorylation was faster when CD43 was ligated with L10 and RaMIG (Fig. 3, lanes 3 and 5). These results show that dimerization of CD43 molecule was sufficient to initiate the transduction of CD43-dependent signals, whereas formation of bigger oligomers resulting in CD43 cross-linking with RaMIG substantially enhanced Vav phosphorylation.

**CD43 Ligation Results in a Transient Association of SLP76 to Vav—**The association of Vav with SLP76 has been reported to play a role in IL-2 gene expression following TcR engagement (11). SLP-76 immunoprecipitates from L10-activated T cell lysates contained tyrosine-phosphorylated Vav (Fig. 4A, lower panel, lanes 1–5); however, when cells were treated with 3D6 no tyrosine-phosphorylated Vav was detected in SLP-76 immunoprecipitates (lanes 6–9). Although we detected a constitutive association between SLP-76 and Vav in control cells (Fig. 4A, lower panel, lanes 6–9), this association was increased following CD43-dependent activation of the cells, reaching a maximum at 5 and 10 min of activation and decreasing after 20 min (lanes 3–5). Despite the fact that equivalent amounts of SLP-76 were immunoprecipitated in all the lanes (data not shown), we found that after 1 min activation, we could barely detect Vav (lane 2, lower panel), although it was highly tyrosine-phosphorylated in those SLP-76 immunoprecipitates (top panel, lane 2). When the reverse experiment was done, that is when we immunoprecipitated Vav from L10- or 3D6-treated T cell lysates (Fig. 4B, lower panel), the association between SLP-76 and Vav was enhanced after 1 min of CD43-specific activation as compared with 3D6-treated cells (Fig. 4B, top panel, compare lanes 2 and 6). These data suggest that the interaction between SLP-76 and Vav is enhanced following CD43 ligation on human T lymphocytes and that it appears to be mediated by tyrosine phosphorylation of Vav.

**CD43-dependent T Cell Activation Induces ERK2 Kinase Activity—**TcR-mediated activation induces Vav tyrosine phosphorylation and ERK2 activation, probably through Vav-SLP-76 association (42). Since CD43 ligation induced Vav tyrosine phosphorylation and Vav-SLP-76 interactions, we tested whether CD43 activated ERK2 kinase activity. The in vitro kinase activity of ERK2 imnmunoprecipitates from CD43-activated T lymphocytes was measured using casein as an exogenous substrate. Fig. 5 shows that CD43 cross-linking induced ERK2 activity in a time-dependent manner (upper panel), reaching a maximum at 5 min (3.5-fold induction over untreated cells, lower panel) and decreasing afterward. This increase in enzymatic activity correlated with ERK2 nuclear translocation. ERK2 was found in the nuclear fraction of T cells stimulated with anti-CD43 mAb L10 (Fig. 6). ERK2 translocation was time-dependent; maximum levels of nuclear ERK2 were found after 5 min activation (lane 3) and decreased after 10 min. ERK2 translocation to the nucleus was specific for CD43 signals since ERK2 was not detected in the nuclear fractions of cells stimulated with the isotype control mAb 3D6 (data not shown).

**CD43 Cross-linking Induces SRE-dependent Luciferase Activity—**Elk-1 is a transcription factor that binds to the serum responsive element (SRE) of the Fos promoter after it is phosphorylated by ERK2 (43). The data presented above suggested that CD43 induction of ERK2 kinase activity could activate Elk-1 transcriptional activity. After 48 h of serum starvation, 3T3-CD43⁺ cells, transiently transfected with the plasmid SRE-luciferase, were stimulated with the anti-CD43 mAb L10, the isotype control mAb 3D6, or 20% FCS for 6 h. As shown in Fig. 7, CD43 ligation induced a 6-fold increase in luciferase activity—
In this study we identified some of the molecules involved in CD43-dependent T cell signaling following Fyn recruitment. The adaptor molecule Shc is one of the tyrosine-phosphorylated proteins observed after CD43-dependent T cell activation (Fig. 1). Shc phosphorylation was time-dependent, and maximum levels were observed after 5 min activation, correlating well with the maximum phosphorylation level of Fyn that was observed after CD43 activation (29). These data and the fact that Shc has been shown to be a substrate for Fyn kinase in T cells (49) suggest that Fyn is probably the kinase that phosphorylates Shc in human T cells in response to CD43 cross-linking.

The formation of macromolecular complexes through tyrosine-phosphorylated sequences and SH2 or phosphotyrosine binding domains allows the activation of other signaling molecules such as phospholipase Cγ or Ras (50). In different cell types, several growth factors have been shown to induce phosphorylation of Shc on tyrosine residues which in turn favors the interaction of Shc with the adaptor molecule GRB2 (51). Here we report that after CD43-specific activation, tyrosine-phosphorylated Shc molecules were found in association with GRB2 (Fig. 1). GRB2 has been reported to interact through its SH3 domains with the GEF Sos (52). Upon T cell activation the interaction of GRB2 and Sos with phosphorylated Shc induces the GTPase activity of Ras, leading to the activation of the Ser/Thr kinase Raf (53) as well as Raf phosphorylation on serine residues. This phosphorylation step induces a change in Raf mobility on SDS-PAGE that correlates with Raf activation. Although it has been demonstrated that CD43 induces cellular proliferation, a function that normally requires Ras activation, we have not been able to detect mobility changes of either Raf or Sos following CD43 cross-linking with the L10 mAb. These data suggest that Shc-GRB2 complexes formed upon CD43 ligation with the L10 mAb in human peripheral T cells are probably not involved in Ras activation. Further experiments need to be performed to determine whether other members of the Raf and Sos families, such as Raf-A or Raf-B and Sos-2, respectively, play a role in Ras activation upon CD43 ligation in human T cells.

Following CD43 cross-linking on the cell surface we observed a protein of approximately 95 kDa phosphorylated on tyrosine. This protein turned out to be the GEF Vav. The phosphorylation levels of Vav increased 4-fold with time after activation, when compared with cells treated with the isotype control mAb (Fig. 2). Vav immunoprecipitates from CD43-activated or resting T lymphocytes contained Shc; although the amount of Shc associated with Vav did not change during activation, its phosphorylation state increased markedly upon activation. Shc-Vav interactions have been reported to occur in vivo (40). Our data suggest that in resting peripheral human T cells, Shc-Vav complexes pre-exist and that following CD43 ligation both proteins become tyrosine-phosphorylated. Vav plays an important role in T and B cell development as well as in receptor-mediated signal transduction (54–56). Since its cloning, Vav was proposed to be a GEF, but the nature of its targets has been controversial. Some reports indicate that Vav could be a GEF for Ras (57). However, recent genetic data provide evidence that Vav is most probably a GEF for Rac 1, another small GTPase that is also activated upon TcR cross-linking (9, 10, 58). Following TcR ligation, Vav is tyrosine-phosphorylated and is able to interact with ZAP-70 kinase and with the adaptor molecule SLP-76 via its SH2 domain. Both interactions play a key role in T cell signaling, since a mutation of Tyr-315 in ZAP-70 abrogates ZAP-70-Vav interaction, resulting in a significant reduction in the tyrosine phosphorylation levels of ZAP-70, Vav, SLP-76, and Shc (59).

FIG. 5. CD43-dependent activation induces ERK2 kinase activity. In vitro kinase assay was performed on ERK2 immunocomplexes isolated from non-stimulated or L10-treated cell lysates, as described under “Materials and Methods.” The reaction mixture was subjected to SDS-PAGE, and radiolabeled products were visualized by autoradiography (top panel). The bottom panel shows the average activity of three independent experiments.

FIG. 6. ERK2 translocates to the nucleus upon T cell CD43-specific activation. 5 × 10⁶ T lymphocytes were activated with L10 mAb for the indicated times. Equivalent amounts of nuclear and cytoplasmic proteins, prepared as described under “Materials and Methods,” were separated by SDS-PAGE and immunoblotted with anti-ERK2 antibodies after transferring to nitrocellulose.

activity as compared with cells treated with the isotype control mAb 3D6. The amplitude of the CD43-mediated signal was comparable to that of 20% FCS. Comparable results were obtained with Jurkat cells (data not shown). CD43 does not induce general gene transcription since after CD43 cross-linking a non-related sequence (see “Materials and Methods”) had basal luciferase activity. Thus, the cell-surface molecule CD43 can initiate a signaling cascade that leads to gene expression, involving the MAPK pathway.

**DISCUSSION**

T cell activation is induced by signals generated through the TcR-CD3 complex and is modified by signals initiated by several cell-surface co-receptors, including CD4, CD8, CD2, CD28, and CD45 among many others (37, 44). The signaling pathways used by these receptor molecules involve the participation of tyrosine phosphorylation cascades initiated by the activation of protein tyrosine kinases such as Lck or Fyn (37). We have previously shown that cross-linking of the CD43 co-receptor molecule on the surface of T lymphocytes leads to the association of CD43 with the protein tyrosine kinase Fyn and to tyrosine phosphorylation of Fyn (29). This event may be the first step in the CD43 signaling pathways that culminates in adhesion (45), IL-2 production (28), cellular proliferation (46), or apoptosis (47, 48).
Overexpression of both SLP-76 and Vav has a synergistic effect on TcR-mediated IL-2 gene expression (42). Here we show that in human peripheral T cells, Vav and SLP-76 are constitutively associated. SLP-76 immunoprecipitations demonstrated the presence of Vav. However, longer exposure of the film was necessary to detect SLP-76 in Vav immunoprecipitates from resting T cells (data not shown), suggesting that Vav-SLP-76 complexes are more stable after SLP-76 immunoprecipitation than after Vav immunoprecipitation. The constitutive association of SLP-76 and Vav could be explained by the fact that basal phosphorylation of SLP-76 has been observed in resting T cells. Our data shows that the Vav-SLP-76 interaction was enhanced by CD43 cross-linking, and the association of tyrosine-phosphorylated Vav and SLP-76 was only detected following CD43 ligation with the L10 mAb (Fig. 4), suggesting that Vav-SLP-76 interactions induced by CD43 signaling may participate in IL-2 gene expression (28).

Vav tyrosine phosphorylation has been implicated in activation of the MAPK pathway. Overexpression of SLP-76 in Jurkat cells augments TcR stimulation of ERK kinase activity (42). T cell lines derived from transgenic mice expressing high levels of Lck activity show constitutive activation of the Ras-Raf-MAPK pathway. Differential activation of small GTPases may play a role in the different cellular responses mediated by CD43.

CD43 dimerization was sufficient to induce Shc and Vav tyrosine phosphorylation. However, the kinetics of this reaction was slower than the kinetics observed in cells that were activated by CD43 cross-linking with a secondary antibody in addition to the L10 mAb (Fig. 3), suggesting that in vivo the interaction of CD43 with any of its ligands may suffice to induce CD43 dimerization and initiate a signaling cascade. CD43 is one of the most abundant cell-surface proteins on T and B cells, protruding from the cell surface to approximately 45 nm (65), making it probable that CD43 is one of the first molecules on the T or B cell that interacts with its ligand(s). Thus generating signals that depend of the CD43 ligand could induce a cellular response and prepare the cell for further signaling from the TcR, the B cell receptor, or other co-receptor molecules.

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