Signal Transduction through the \(\beta 1\) Integrin Family Surface Adhesion Molecules VLA-4 and VLA-5 of Human B-cell Precursors Activates CD19 Receptor-associated Protein-tyrosine Kinases

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We demonstrate that the CD19 receptor associates with the \(\beta 1\) family integrin receptors on human B-cell precursors as well as mature B-lymphocytes, and engagement of the \(\beta 1\) family integrin receptors with monoclonal antibody homoconjugates leads to rapid activation of the CD19-associated protein-tyrosine kinases (PTK) and results in hyperphosphorylation of CD19 on tyrosine residues. Our findings prompt the hypothesis that homocoujugated-induced integrin clustering may effect the immunoreceptor tyrosine-based activation motiff (ITAM) and/or VLA-5 generates pleiotropic biologic responses has not been deciphered. However, recent studies demonstrated that signaling through these adhesion receptors leads to enhanced tyrosine phosphorylation of multiple protein substrates, suggesting the involvement of protein-tyrosine kinases (PTK) in the generation of physiologically significant biochemical events after the ligation of the \(\beta 1\) family integrin receptors (4, 22–28).

Recent studies indicated that the CD19 receptor may be a common response element involved with PTK signaling stimulated through diverse B-cell surface molecules, such as the B-cell antigen receptor, CD40 receptor, and CD72 receptor (29). Here, we present experimental evidence that 1) the CD19 receptor is physically associated with the \(\beta 1\) family integrin receptors on human BCP as well as mature B-lymphocytes, and 2) engagement of the \(\beta 1\) family integrin receptors leads to rapid activation of the CD19-associated PTK and results in hyperphosphorylation of CD19 on tyrosine residues. The physical association between CD19 and \(\beta 1\) family integrins suggests that homocoujugate-induced integrin clustering may effect the approximation, activation of the CD19-associated PTK by intermolecular cross-phosphorylation, and subsequent tyrosine phosphorylation of the CD19 receptor. The ability of the \(\beta 1\) family integrin receptors to transmit a biochemical signal triggering the CD19-linked multifunctional PTK pathway provides a possible explanation for the pleiotropic biologic responses generated through adhesive VLA-4 and VLA-5-mediated contacts.

Integrins are heterodimeric integral plasma membrane proteins that mediate cell-cell as well as cell-extracellular matrix adhesion (1–4). All members of the integrin superfamly exist as \(\beta\)-heterodimers. The \(\beta 1\) integrin common chain CD29 can associate with distinct \(\alpha\) chains to form the very late antigens (VLA), which serve as receptors for extracellular matrix proteins (1–4). Members of the VLA \(\beta 1\) integrin subfamily of adhesion molecules are thought to play a pivotal regulatory role in human B-cell ontogeny by mediating cell-cell and cell-stroma interactions of B-cell precursors (BCP) (1, 5–15). Two members of the \(\beta 1\) or VLA integrin family expressed on normal and leukemic BCP are VLA-4 (heterodimer of \(\beta 1\)CD29 subunit with \(\alpha 4\)CD49d subunit) and VLA-5 (heterodimer of \(\beta 1\)CD29 subunit with \(\alpha 5\)CD49e subunit) (5–15). VLA-4 binds to vascular adhesion molecule-1 (VCAM-1) on stromal cells, and it recognizes three distinct cell adhesion sites in the COOH-terminal heparin binding domain and in adjacent alternatively spliced IIIICS domain of fibronectin, which is the major component of the extracellular matrix in lymphohematopoietic microenvironments (16–21). VLA-5 binds to the RGD motif in the central cell binding domain located in the 10th type-111 repeat in A and B subunits of fibronectin (3, 13). In addition to mediating cell adhesion, the engagement of \(\beta 1\) family integrin molecules influence several other important cellular events (1–4). The molecular mechanisms by which the engagement of VLA-4 and/or VLA-5 generates pleiotropic biologic responses has not been deciphered. However, recent studies demonstrated that signaling through these adhesion receptors leads to enhanced tyrosine phosphorylation of multiple protein substrates, suggesting the involvement of protein-tyrosine kinases (PTK) in the generation of physiologically significant biochemical events after the ligation of the \(\beta 1\) family integrin receptors (4, 22–28).

EXPERIMENTAL PROCEDURES

CdL Lines—We used the pre-B (CD19−CD10+CD4+Slg−) cell line NALM-6 and the Epstein-Barr virus-transformed mature B-cell line line AK to study signal transduction through the VLA-4 and VLA-5 receptors. Antibodies and Antibody Homocoujugates—15G8 (anti-VLA-4) and SAM-1 (anti-VLA-5) were purchased from Research Diagnostics, Inc. (Flanders, N.J.). B43 (anti-CD19) monoclonal antibody (mAb) was produced in the Uckun Laboratory, as described previously (31). The reactivity of these antibodies with their target receptors was determined by

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1The abbreviations used are: VLA, very late antigens; BCP, B-cell precursor(s); PTK, protein-tyrosine kinases(s); mAb, monoclonal antibody; GST, glutathione S-transferase; PBS, phosphate-buffered saline; APT, anti-phosphotyrosine; FITC, fluorescein isothiocyanate.
two-color immunofluorescence and multiparameter flow cytometry, as described (31). Polyclonal anti-CD19 for Western blot analyses was produced by immunization of rabbits with a glutathione S-transferase (GST)-CD19 fusion protein containing the amino acids 410–540 of the CD19 cytoplasmic domain. Fab₂ goat anti-human IgM was purchased from Jackson Immunologicals. Antibodies against the Src PTK family members were obtained from rabbit immunized with synthetic peptides representing the unique domains of the individual enzymes (32). Rabbit anti- phosphotyrosine antibodies were generated against a copolymer of phosphotyrosine, glycine, and alanine coupled to keyhole limpet hemocyanin (kindly provided by Gary Schieve, Oncogen, Seattle). Polyclonal anti-Syk was raised against a GST-Syk fusion protein containing the first 150 amino acids of Syk (33). Polyclonal anti-Btk was produced by immunization of rabbits with a GST-Btk fusion protein containing the first 150 amino acids of Btk (34). mAb homologues were prepared as described previously (29). In brief, reactive sulfhydryl groups were introduced into the first mAb by mixing it at a concentration of 4 mg/ml in PBS, pH 7.5, with a 5-fold molar excess of 2-iminothiolane (Pierce), prepared immediately before use as a 10 mM solution in 50 mM sodium phosphate buffer, pH 8.2. The second antibody was derivatized with a 5-fold molar excess of sulfo-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC; Pierce), freshly prepared as an 8 mM solution in PBS, pH 7.5. Both modification reactions were allowed to proceed for 2 h at room temperature with gentle rocking in sterile endotoxin-free glass vials (Miles, West Haven, CT). Sephadex G-25 PD10 prepacked columns (Pharmacia Biotech Inc.) were used to separate the derivatized monoclonal antibodies from low molecular weight reaction products and excess reagents, as described (31). Derivatized antibodies were then conjugated as described (31, 35). All homologues used in the present study had a molecular mass of 360 kDa, consistent with a two-covalently coupled IgG (150 kDa each) molecules.

Anti-phosphotyrosine Immunoblot Analysis—Phosphorylation of proteins on tyrosine residues was measured at 10, 20, 30, 45, 1 min, 2 min, 3 min, 5 min, 10 min, and/ or 20 min after ligation of various B-cell surface receptors by immunoblotting with a highly specific polyclonal anti-phosphotyrosine (APT) antibody, as described previously (29). Whole cell lysates were prepared by pelleting 1.0 ml of cell suspension for 8 min in a microcentrifuge, aspirating the supernatant, and adding 150 μl of sodium dodecyl sulfate (SDS) lysis buffer (50 mM Tris-HCl, 2% SDS, 10% glycerol) containing 100 μM sodium vanadate and 25 mM dithiothreitol, as reported previously (35, 36). Each sample was boiled for 5 min. The DNA was sheared by several passages through a 28-gauge needle and equivalent amounts of protein in 40 μl of SDS reducing sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 100 μM Na₃VO₄, and 25 mM dithiothreitol) were loaded onto 10.5% SDS-polyacrylamide gels and electrophoresed overnight at 4 mA. The proteins were transferred to a 0.45-μm Immobilon polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA) for 1 h at 130 mA using a semidy transfer apparatus (Hoerter Scientific Instruments, San Francisco, CA). The polyvinylidene difluoride membranes were incubated in 35 μg/ml purified rabbit anti-phosphotyrosine antibody in blocking solution (0.9% NaCl, 10 mM Tris, 0.01% NaN₃, pH 7.2, 5% bovine serum albumin, 1% ovalbumin) for 2 h at room temperature, followed by two 10-min washes in rinsing buffer. Blots were incubated with 1 μCi/ml 125I-labeled protein A (specific activity = 30 μCi/μg ICN Biomedical) in blocking solution. After a 30-min incubation in 125I-protein A, blots were washed three times in rinsing buffer (0.9% NaCl, 10 mM Tris-HCl, 0.01% NaN₃, pH 7.2), dried, and autoradiographed using a XAR-5 film (Eastman Kodak Co.). Prestained molecular weight markers (Amer sham Corp.) were included on each gel as standards.

To study tyrosine phosphorylation of CD19 receptor, CD19 receptor was immuno precipitated using the anti-CD19 mAb B43 from whole cell lysates of B-lineage lymphoid cells following the engagement of various B-cell surface receptors. In brief, following various stimulation regimens, cells (5 × 10⁶ cells/sample) were solubilized in 0.5 ml of 1% Nonidet P-40 lysis buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, plus 1 mM EDTA) containing 0.1 mM sodium orthovanadate, 1 mM sodium molybdate as phosphatase inhibitors, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride as protease inhibitors on ice for 30 min. Lysates were spun twice at 12,000 × g for 15 min at 4 °C prior to immunoprecipitation. 100 μg of cell lysates were immunoprecipitated with 3 μg of B43 antibody for 90 min at 4 °C. The immune complexes were collected with 50 μl of a 1:1 (v/v) slurry of protein A-Sepharose (Sigma) in Nonidet P-40 buffer. The immunoprecipitates were washed four times with Nonidet P-40 buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 1% (v/v) Nonidet P-40, 1 mM Na₃VO₄, 1 mM sodium molybdate, 1 mM phenylmethylsulfonyl fluoride, 0.01% NaN₃, and 0.01% SDS) at 4 °C. The pellets were resuspended in 100 μl of 2× SDS reducing sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.01% NaN₃, 0.01% leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride) and boiled for 5 min. The samples were subjected to SDS-polyacrylamide gel electrophoresis and subsequently immunoblotted using monoclonal and polyclonal anti-CD19 (5 μg/ml) antibodies, using a previously detailed immunoblotting procedure (29). To study tyrosine phosphorylation of Lyn kinase and Lyn kinase-associated protein substrates, we immunoprecipitated Lyn from Nonidet P-40 lysates of NALM-6 cells (200 μg of cell lysates/sample) with a rabbit anti-Lyn antibody (2 μg/200 μg of lysate) and subjected the Lyn immune complexes to APT Western blot analysis, as described (29). 125I-Labeled protein A (1 μCi/ml; specific activity = 30 μCi/μg ICN Biomedical) was used to detect APT reactive or anti-CD19 reactive proteins in the CD19 or Lyn immune complexes. After a 30-min incubation with 125I-labeled protein A, blots were washed three times in rinsing buffer, dried, and autoradiographed using a XAR-5 film (Eastman Kodak Co.).

Immune Complex Kinase Assays—Immune-complex protein kinase assays were performed as described (29, 37). 200 μg of Nonidet P-40 lysates/sample were immunoprecipitated with a rabbit anti-Lyn antibody (2 μg/200 μg of lysate), rabbit anti-Syk antibody (2 μg/200 μg of lysate), or APT antibody (5 μg/200 μg of lysate), as reported previously (29).

RESULTS

Cross-linking β1 Integrin Family Surface Adhesion Molecules VLA-4 and VLA-5 Induces Enhanced Tyrosine Phosphorylation of the CD19 Receptor—Recent studies demonstrated that signaling through these adhesion receptors leads to enhanced tyrosine phosphorylation of multiple protein substrates, suggesting the involvement of PTK in the generation of physiologically significant biochemical events after the ligation of the β1 family integrin receptors (4, 22–28). The CD19 receptor-linked signal transduction pathway in human B-cell precursors and mature B-cells is coupled via tyrosine phosphorylation to multiple functionally important receptors, as shown previously (29). Virtually, 100% of the B-lineage lymphoid cells...
expressing VLA-4 or VLA-5 receptors also express the CD19 receptor on their surface (Fig. 1). We therefore hypothesized that the CD19-mediated and VLA-4- or VLA-5-mediated PTK signals may share common substrates. To test this hypothesis, we compared the profiles of tyrosine-phosphorylated proteins in whole cell lysates prepared at various time points after cross-linking of either the CD19 receptor or the β1 integrin family adhesion receptors VLA-4 and VLA-5 by Western blot analysis using a polyclonal antibody specific for phosphotyrosine (APT). Fig. 2A illustrates that cross-linking of the VLA-4 or VLA-5 on NALM-6 pre-B-cells with a monoclonal antibody homocoujugate in rapid increase in tyrosine phosphorylation of multiple electrophoretically distinct phosphoprotein substrates with apparent molecular masses of 55, 72, 76, 95, and 120 kDa. By comparison, no increase in tyrosine phosphorylation was noted in these sIgM negative pre-B-cells after stimulation with an anti-IgM antibody homocoujugate (Fig. 2A). Similar results were obtained with an Epstein-Barr virus-transformed mature B-cell line (Fig. 2B). In both NALM-6 pre-B-cells and AK mature B-cells, the profile of APT reactive protein substrates after cross-linking of the VLA-4 or VLA-5 molecules with appropriate monoclonal antibody homocoujugates was essentially identical to that generated by the engagement of the CD19 receptor with an anti-CD19 × CD19 homocoujugate, suggesting that these receptors are linked to the same PTK regulatory pathway. In accordance with our previous report, the engagement of the antigen receptor on AK mature B-cells with an anti-IgM antibody triggered tyrosine phosphorylation of the same substrates as the CD19 engagement.

The CD19 receptor is physically and functionally associated with Src family PTK, of which Lyn kinase is the predominant member in human pre-B-cells and mature B-lymphocytes (29). The engagement of the CD19 receptor with a high affinity anti-CD19 monoclonal antibody or its homocoujugate rapidly activates the associated PTK and results in tyrosine phosphorylation of CD19 (29). In whole cell lysates of pre-B-cells as well as mature B-cells, a 95-kDa phosphoprotein substrate showed rapid and strong tyrosine phosphorylation after engagement of the β1 integrin family adhesion receptors VLA-4 or VLA-5 (Fig. 2). Based on its molecular mass, we hypothesized that the 95-kDa tyrosine phosphorylated protein could be the CD19 receptor, which has been shown to become tyrosine-phosphorylated following engagement of various B-cell surface receptors (29, 30). To test this hypothesis, we subjected CD19 immune complexes from the lysates of NALM-6 pre-B-cells, which were stimulated with an anti-CD19 × VLA-5 × CD19 homocoujugate, to APT Western blot analysis. As shown in Fig. 3A, left panel, CD19 showed increased tyrosine phosphorylation within 1 min after VLA-5 cross-linking. The abundance of the CD19 receptor protein, as estimated by immunoblotting, did not change during the course of the experiment (Fig. 3A, right panel). These results indicated that activation of CD19-associated PTK, cross-linking of the VLA-4 receptor on NALM-6 cells with an anti-VLA-4 × VLA-4 homoconjugate also resulted in rapid tyrosine phosphorylation of the CD19 receptor (data not shown). Similarly, CD19 receptor of AK mature B-cells showed increased tyrosine phosphorylation within 5 min after VLA-5 cross-linking (Fig. 3B, left panel) or VLA-4 cross-linking (data not shown).

Lyn is the predominant PTK associated with the CD19 receptor in B-lineage lymphoid cells (29, 30). To obtain preliminary information about the possibility that the increased tyrosine phosphorylation of CD19 after VLA-5 cross-linking could be due to increased PTK activity of the CD19-associated Lyn kinase, Lyn immune complexes from Nonidet P-40 lysates of AK cells were subjected to APT Western blot analysis. As shown in Fig. 3B, right panel, engagement of the VLA-5 receptor resulted in increased tyrosine phosphorylation of Lyn protein within 5 min. Concomitant with enhanced tyrosine phosphorylation of Lyn between 5 and 20 min after VLA-5 cross-linking, CD19 became detectable in the Lyn immune complexes as a tyrosine phosphorylated protein substrate (Fig. 3B, right panel). These results indicated that activation of CD19-associated PTK, such as Lyn, may be an integral component of the signal transduction cascade triggered by β1 integrin family adhesion receptors.

β1 Integrin Family Surface Adhesion Molecules VLA-4 and VLA-5 Are Physically Associated with the CD19 Receptor and Their Cross-linking Stimulates the Enzymatic Activity of CD19-associated PTK—CD19 has been shown to be capable of forming functionally important protein complexes on the surface of...
Signaling through VLA-4 and VLA-5

Fig. 3. Tyrosine phosphorylation of CD19 upon cross-linking of VLA-4 or VLA-5 receptors. NALM-6 pre-B cells and AK mature B cells were stimulated with an anti-VLA-5 or anti-VLA-4 mAb homogenous (1 μg/ml) for the indicated periods to engage and dimerize the respective target receptors. Subsequently, CD19 receptor was immunoprecipitated from Nonidet P-40 lysates, and equal amounts were subjected either to APT Western blot analysis (left panel of A and B) or to anti-CD19 Western blot analysis (right panel of A), as described under “Experimental Procedures.” In parallel, LYN immunoprecipitates from Nonidet P-40 lysates of AK mature B cells stimulated with anti-VLA-5 or anti-VLA-4 mAb homogenous (1 μg/ml) were also examined by APT Western blot analysis for tyrosine phosphorylation of LYN and LYN-associated kinase substrates (B, right panel). Arrowheads indicate the position of CD19.

Linking VLA-4 or VLA-5. For these experiments, mature B cells were stimulated with anti-VLA-4 × VLA-4 or anti-VLA-5 × VLA-5 homogenous, cell lysates were immunoprecipitated with anti-CD19 mAb B43, and immune complex kinase assays were performed. As shown in Fig. 4B, the kinase activity of the CD19 immune complexes was enhanced in a time-dependent manner following stimulation with anti-VLA-4 × VLA-4 or anti-VLA-5 × VLA-5. The immunoprecipitated CD19 molecule was also phosphorylated during the kinase reactions (Fig. 4B).

After engagement of the CD19 receptor Src family PTK, of which Lyn is the predominant member in pre-B as well as mature B cells, they are activated and phosphorylated on tyrosine residues. Lyn kinase is also activated after CD19 ligation and associates with the tyrosine-phosphorylated CD19 receptor. Lyn kinase has been shown to associate with integrin-dependent cytoskeletal complexes in platelets and fibroblasts. Lyn kinase has also been shown to associate with integrin-dependent cytoskeletal structures and to become activated in platelets after engagement of integrins. To examine the activation of the Lyn and Syk kinases, both of which are intimately linked to the CD19-linked signal transduction pathway, by signaling through a1 family integrin receptors, VLA-4 and VLA-5, were cross-linked to the surface of NALM-6 pre-B cells and AK mature B cells by addition of the appropriate homogenous conjugates, and the PTK activities of Lyn and Syk were estimated by immune complex kinase

human B-lineage lymphoid cells (29, 30, 38). Cross-linking of the CD19-associated surface receptors may effect the approximation and, by intermolecular cross-phosphorylation, activation of the CD19-associated PTK and subsequent tyrosine phosphorylation of the CD19 receptor. The ability of the a1 family integrin receptors to transmit a biochemical signal leading to hyperphosphorylation of the CD19 receptor on tyrosine residues prompted the hypothesis that a physical association may exist between CD19 and a1 family integrins. To test this hypothesis, VLA-4 and VLA-5 immune complexes from NALM-6 pre-B cells were subjected to Western blot analysis with an anti-CD19 antibody. As shown in Fig. 4A, CD19 was detected in both the VLA-4 and VLA-5 immune complexes.

CD19 is associated with Src family PTK, including Lyn, in resting pre-B and mature B cells. To explore the possibility that homogenous-induced integrin clustering might activate the CD19-associated PTK, we first examined whether the PTK activity in CD19 immunoprecipitates is increased after cross-linking VLA-4 or VLA-5. For these experiments, mature B cells were stimulated with anti-VLA-4 × VLA-4 or anti-VLA-5 × VLA-5 homogenous, cell lysates were immunoprecipitated with anti-CD19 mAb B43, and immune complex kinase assays were performed. As shown in Fig. 4B, the kinase activity of the CD19 immune complexes was enhanced in a time-dependent manner following stimulation with anti-VLA-4 × VLA-4 or anti-VLA-5 × VLA-5. The immunoprecipitated CD19 molecule was also phosphorylated during the kinase reactions (Fig. 4B). As reported previously, the major phosphoprotein species associated with CD19 migrated in the 50–60-kDa region. Notably, a closely spaced 53/56-kDa doublet, most likely corresponding to the autophasophorylated products of the two alternatively spliced LYN transcripts, was induced by engagement of the VLA-5 receptor but not the VLA-4 receptor.

After engagement of the CD19 receptor Src family PTK, of which Lyn is the predominant member in pre-B as well as mature B cells, they are activated and phosphorylated on tyrosine residues (29). Syk kinase is also activated after CD19 ligation and associates with the tyrosine-phosphorylated CD19 receptor. Syk kinase has been shown to associate with integrin-dependent cytoskeletal complexes in platelets and fibroblasts (4). Syk kinase has also been shown to associate with integrin-dependent cytoskeletal structures and to become activated in platelets after engagement of integrins (4). To examine the activation of the Lyn and Syk kinases, both of which are intimately linked to the CD19-linked signal transduction pathway, by signaling through a1 family integrin receptors, VLA-4 and VLA-5, were cross-linked to the surface of NALM-6 pre-B cells and AK mature B cells by addition of the appropriate homogenous conjugates, and the PTK activities of Lyn and Syk were estimated by immune complex kinase activity in CD19 immunoprecipitates is increased after cross-linking VLA-4 or VLA-5.

Fig. 4. Physical and functional associations between a1 family surface adhesion receptors and CD19. A, VLA-4 and VLA-5 receptors were immunoprecipitated from Nonidet P-40 lysates of unstimulated NALM-6 pre-B cells (10 × 10⁶ cells/sample), and the immune complexes were subjected to anti-CD19 Western blot analysis, as described under “Experimental Procedures.” No IAb indicates mock-immunoprecipitated lysates to which no primary antibody was added. B, AK mature B cells (10 × 10⁶ cells/sample) were stimulated with homogenous conjugates (1 μg/ml) of anti-VLA-4 or anti-VLA-5 monoclonal antibodies for the indicated periods and then lysed in Nonidet P-40 buffer. Equal amounts of lysate (200 μg of protein/reaction mixture) were immunoprecipitated with B43 (anti-CD19) mAb (6 μg/200 μg of lysate protein), and immune complex protein kinase assays were performed, as described under “Experimental Procedures.” Molecular masses (in kilodaltons) of the phosphoprotein substrates were calculated from prestained molecular size markers run as standards. Arrowheads indicate the position of CD19.

CD19 is associated with Src family PTK, including Lyn, in resting pre-B and mature B cells. To explore the possibility that homogenous-induced integrin clustering might activate the CD19-associated PTK, we first examined whether the PTK activity in CD19 immunoprecipitates is increased after cross-linking VLA-4 or VLA-5. For these experiments, mature B cells were stimulated with anti-VLA-4 × VLA-4 or anti-VLA-5 × VLA-5 homogenous, cell lysates were immunoprecipitated with anti-CD19 mAb B43, and immune complex kinase assays were performed. As shown in Fig. 4B, the kinase activity of the CD19 immune complexes was enhanced in a time-dependent manner following stimulation with anti-VLA-4 × VLA-4 or anti-VLA-5 × VLA-5. The immunoprecipitated CD19 molecule was also phosphorylated during the kinase reactions (Fig. 4B). As reported previously, the major phosphoprotein species associated with CD19 migrated in the 50–60-kDa region. Notably, a closely spaced 53/56-kDa doublet, most likely corresponding to the autophasophorylated products of the two alternatively spliced LYN transcripts, was induced by engagement of the VLA-5 receptor but not the VLA-4 receptor.

2 F. M. Uckun, unpublished observations.
assays. As shown in Fig. 5, homoconjugate-induced dimerization of the VLA-5 receptor caused rapid activation of Lyn and Syk kinases in both NALM-6 and AK cells. By comparison, VLA-4 cross-linking did not significantly stimulate the Lyn kinase in either cell line (Fig. 6). Unlike Lyn, Syk kinase was activated following engagement of not only the VLA-5 receptor, but the VLA-4 receptor as well (Fig. 6).

**DISCUSSION**

CD19 is a physiologically important multifunctional surface receptor, which is expressed throughout the B-cell ontogeny. It is physically and functionally associated with Src family PTK to form transmembrane receptor tyrosine kinases with ancillary signal-transducing functions (29, 30). Src family PTK in these CD19-PTK complexes act as signal transducers and cou-
CD19 to downstream cytoplasmic signaling pathways (29, 30). CD19 has been shown to play an important coreceptor role in optimal signal transduction through the B-cell antigen receptor (29, 30). More recent studies demonstrated that the CD19 signals can complement and modulate the signals through several other B-cell receptors as well, indicating that the co-receptor role of CD19 is not restricted to the B-cell antigen receptor (29, 30). CD19 may serve as an ancillary signal transduction unit that facilitates an effective communication between different B-cell receptors for the generation of an optimal B-cell immune response (29, 30). Recent studies in CD19-deficient mice demonstrated that CD19 is very important for initial B-cell activation by T-cell-dependent antigens and development of a memory cell compartment (39). Furthermore, CD19 appears to be required for antigen-driven expansion and maintenance of B-1 subset in the B-cell compartment (39). Other studies have indicated that the membrane-associated CD19 receptor-Lyn enzyme complex serves as a regulator of apoptosis in human B-cell precursors (37) as well as mature B-cells (31). The results presented herein regarding the physical and functional association of the CD19 receptor with β1 integrin family adhesion receptors extend these earlier studies and offer the first insights into the role of the CD19 coreceptor and CD19-associated PTK in signal transduction through the VLA-4 and VLA-5 receptors.

VLA-4 mediates cell-cell interactions in the contexts of homing (3), homotypic aggregation (40, 41), and cognate interactions between B and T cells (42). For example, VLA-4 functions as a homing receptor on both mouse and human lymphocytes, facilitating their attachment to Peyer’s Patch high endothelial venule cells (3, 41). Ryan et al. (7, 8) reported that VLA-4 interaction with its ligand VCAM-1 mediates the adhesion of human B-cell to cultured bone marrow stroma cells. Similarly, Dittel et al. (9) found that IL-7 responsive human B-cell adhere to bone marrow stroma via the VLA-4/VCAM-1 interactions, reminiscent of the observations by Miyake et al. (43) that VLA-4 on mouse pre-B-cells mediates their adhesion to bone marrow stroma. Leukemic B-cell have been shown to bind to purified fibronectin via VLA-4 and VLA-5 (12). According to the findings of Bradstock et al. (11) the adhesion of leukemic B-cell to bone marrow fibroblasts occurs predominantly through interaction of VLA-4 with VCAM-1. Furthermore, the ability of leukemic B-cell to migrate into stroma depends on β1 integrin expression (10, 13). Previous studies demonstrated that treatment of leukemic B-cell with antibodies to β1 common subunit or a mixture of anti-VLA-4 and anti-VLA-5 antibodies abrogates their ability to migrate into bone marrow stroma (13, 44). Notably, infusion of a blocking antibody to the α4 subunit of the VLA-4 receptor in primates has been shown to cause a 200-fold increase in circulating hematopoietic progenitor cells, implicating the VLA-4/α4 in the regulation of the in vivo migration and trafficking of hematopoietic progenitor cells (45).

Here, we presented experimental evidence that CD19 receptor is physically associated with the β1 family integrin receptors on human B-cell as well as mature B-lymphocytes and engagement of the β1 family integrin receptors leads to rapid activation of the CD19-associated PTK and results in hyper-phosphorylation of CD19 on tyrosine residues. The physical association between CD19 and β1 family integrins prompts the hypothesis that homoconjugate-induced integrin clustering may effect the approximation and, by intermolecular cross-phosphorylation, activation of the CD19-associated PTK and subsequent tyrosine phosphorylation of the CD19 receptor. The ability of the β1 family integrin receptors to transmit a biochemical signal leading to activation of the CD19-linked PTK pathway, which is coupled to other functionally important receptors as well as several distal second messengers (29, 30), provides an explanation for the pleiotropic responses generated through adhesion contacts mediated by VLA-4 and VLA-5.
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