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c-Abl Is Involved in the F-Actin Assembly Triggered by L-Selectin Crosslinking

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L-selectin is a cell adhesion molecule mediating the initial capture and subsequent rolling of leukocytes along the endothelial cells expressing L-selectin ligands. In addition to its action in adhesion, an intracellular signaling role for L-selectin has been recognized. Its cytoplasmic domain is involved in signal transduction following antibody crosslinking and in the regulation of receptor binding activity in response to intracellular signals. In this work, we demonstrated that L-selectin crosslinking led to F-actin polymerization and redistribution in human neutrophils. Using immuno-fluorescence microscopy, we observed that F-actin redistribution spatiotemporally related to the polarization of L-selectin. STI571, a specific inhibitor for cytoplasmic tyrosine kinase c-Abl, can inhibit F-actin polymerization and c-Abl redistribution in the activated neutrophils. Furthermore, we determined that c-Abl redistributed to the region where L-selectin polarized and associated with L-selectin in the activated neutrophils. The association between L-selectin and c-Abl was reduced by cytochalasin B. These results suggested that c-Abl was involved in the F-actin alteration triggered by L-selectin crosslinking in human neutrophils.

Key words: c-Abl kinase, cross-linking, F-actin reorganization, L-selectin, protein association.

Recruitment of leukocytes from blood into tissues is controlled by a variety of adhesion molecules on the surface of the endothelium and circulating leukocytes (*1*). L-selectin which belongs to selectin family is one of the adhesion molecules. It constitutively expresses on leukocytes and plays a fundamental role in the immune system by mediating lymphocyte homing and recruitment of leukocytes to inflammatory sites (*2, 3*).

Except for its adhesive functions, many reports suggested that L-selectin can act as a signal transduction molecule. Transmembrane signaling *via* L-selectin could enhance the microvascular sequestration of neutrophils at sites of inflammation (*4*) and increase the neutrophil adhesive functions *via* β2-integrin (*5*). Clustering of L-selectin by antibody crosslinking led to a rapid induction of actin assembly and CD18 co-localization with L-selectin in the plasma membrane (*6, 7*). Crosslinking of human L-selectin with monoclonal antibody mobilized intracellular Ca^{2+} (*8, 9*), increased tumor necrosis factor α and interleukin 8 mRNA expression (*8*), induced O^{2-} generation (*10*), enhanced some proteins phosphorylation (*11–13*). Crosslinking of L-selectin on lymphocytes or Jurkat cells with anti–L-selectin monoclonal antibody initiated a signaling cascade from L-selectin *via* tyrosine kinase $p56^{lck}$, Grb_2/Sos, Ras, MAPK, and Rac_2, resulting in O^{2-} generation and actin polymerizatioin (*14, 15*). L-selectin engagement in T lymphocytes resulted in a src-tyrosine kinase dependent activation of NFAT, small G-proteins (Ras and Rac1/2), MAP-kinase (ERK1/2) and JNK (*16, 17*). Previous studies have indicated that engagement of L-selectin resulted in the activation of signaling pathways that were dependent on both reorganization of structural cytoskeleton and kinase activity.

c-Abl is a nonreceptor tyrosine kinase ubiquitously expressed and contains a catalytic domain, polyproline rich regions, and SH2 and SH3 domains that are involved in protein–protein interactions and may also regulate the kinase activity. c-Abl could regulate actin cytoskeleton in different activated cell lines (*18–21*). In this article, we showed that the kinase activity of c-Abl was greatly increased and it was spatially reclined to the region where F-actin and L-selectin concentrated in the activated neutrophils. Furthermore, we also observed that c-Abl was co-immunoprecipitated with L-selectin in a F-actin dependent manner. These data suggested that c-Abl was involved in the F-actin alteration induced by L-selectin engagement.

MATERIALS AND METHODS

Reagents and Antibodies—Dextran T-500 and Glutathione sepharose $4B^{TM}$ (17-0756-01) were purchased from Amersham Biosciences. W6/32 (the anti-human HLA-ABC monoclonal antibody, mouse IgG) was purchased from eBioscence, Inc. DREG56 (the anti–L-selectin monoclonal antibody, mouse IgG_1, sc-18851), H-300 (the anti–c-Abl polyclonal antibody, rabbit IgG, sc-13076), K12 (the anti–c-Abl monoclonal antibody, rabbit IgG, sc-131), PY99 (the anti-phosphotyrosine monoclonal antibody, mouse IgG_{2b}, sc-7020), non-conjugated $F(ab')_2$ fragment of goat anti-mouse IgG (sc-3696), FITC-conjugated $F(ab')_2$ fragment of goat anti-mouse IgG (sc-3699) and TRITC-conjugated $F(ab')_2$ fragment of goat anti-mouse

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IgG (sc-3796) were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). 8E9 (the anti–c-Abl polyclonal antibody, mouse IgG) was purchased from BD biosciences pharmingen. FITC-conjugated goat anti-rabbit IgG (H+L) and TRITC-conjugated goat anti-rabbit IgG (H+L) were from Jackson Laboratory (Bar Harbor, ME). Cytochalasin B, FITC-conjugated Phalloidin, G7781 (the anti–glutathine-S-transferase, rabbit IgG) were purchased from Sigma. STI571, an inhibitor to non-receptor tyrosine kinase c-Abl, was a gift of Novartis Pharma schweiz AG (Basel, Switzerland).

Neutrophil Isolation—Neutrophils were isolated by standard procedures after sedimentation of erythrocytes by dextran T-500 and centrifugation of leukocytes over Ficoll-hypaque gradients (*22*). After lysis of contaminating erythrocytes with hypotonic saline (0.17 M Tris, 0.16 M NH_4Cl, pH 7.2), neutrophils were washed and resuspended in cold PBS containing 0.1% bovine serum albumin (BSA). The isolated neutrophils were stored at 4°C until use. More than 95% of the isolated cells were polymorphonuclear leukocytes, and viability was determinate to be >98% by trypan blue exclusion.

GST-Crk II-CTD Proteins Purification—E. coli strain BL-21 (DE3) transformed with GST-CrkII-CTD expression vectors were incubated in 200 ml LB culture medium with 50–100 μg/ml ampicillin and induced with 0.3 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3 h at 37°C. Cells were harvested in lysis buffer (20 mM HEPES, pH 7.5, 120 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM PMSF, 10 μg/ml each of aprotinin and leupeptin) and disrupted by sonication. Lysates were cleared by centrifugation at 15,000 × *g* at 4°C for 30 min. The supernatants were applied to a GSH-sepharose column. After extensive washing with the lysis buffer, the isolated proteins were stored at 4°C for experiments (not more than 1 wk).

Cell Stimulation—Neutrophils were resuspended in PBS with 0.5% BSA and 20 mM glucose, ligated with DREG56 at a final concentration of 10 μg/ml at 4°C for 20 min alone or followed interacting with F $(ab')_2$ fragment of goat anti-mouse IgG at a final concentration of 20 μg/ml at 4°C for another 20 min, and then incubated at 37°C for 10 min to make L-selectin polarization. In some other experiments, to detect the distribution of L-selectin on the surface of neutrophils before or after antibody crosslinking, non-fluorescent secondary antibody was replaced by FITC- or TRITC-conjugated $F(ab')_2$ fragment of goat anti-mouse IgG. To test the effect of cytochalasin B or STI571 on the redistribution of c-Abl, cells were preincubated with cytochalasin B (100 μM) or STI571 (10 μM) at 4°C for 30 min before crosslinking with primary and secondary antibody. To investigate the c-Abl kinase activity, cells were washed with sterile Hepes saline (H/S; 132 mM NaCl, 20 mM Hepes, 5 mM KCl, 1 mM $CaCl_2$, 0.7 mM $MgCl_2$, 0.8 mM $MgSO_4$) and stimulated with DREG56 at 37°C for 5 min.

Flow Cytometry—The quantification of polymerized F-actin within neutrophils was performed by flow cytomery. Briefly, the resting or stimulated neutrophils were fixed, permeabilized, and then stained with 3.3×10^{-7} M FITC-conjugated phalloidin at room temperature for 20 min and washed with PBS. Cells were examined on a FACScan, and values were expressed as relative fluorescence index (RFI). For those inhibitor-blocking experiments, the inhibitor (STI571) was incubated with cells at 4°C for 30 min before L-selectin crosslinking.

Immunofluorescence Microscopy—To detect the influence of antibody crosslinking on F-actin assembly, after ligation of L-selectin with DREG56 and non-conjugated secondary antibody, neutrophils were fixed with 1% paraformaldehyde for 15 min at room temperature, permeabilized with 0.2% Triton X-100 in PBS (containing 5 mM EDTA and 2% FBS) for 5 min. After washing with PBS, cells were stained with 3.3×10^{-7} M FITC-conjugated phalloidin at room temperature for 20 min and washed. To investigate the localization of nonreceptor tyrosine kinase c-Abl in resting and activated neutrophils, after crosslinking with DREG56 and FITC-conjugated secondary antibody, neutrophils were fixed, permeabilized and then incubated with anti–c-Abl polyclonal antibody (H-300, 10 μg/ml), washed with PBS, and incubated with TRITC-conjugated goat anti-rabbit IgG (H+L) for another 30 min, then washed with PBS. To dually label F-actin and c-Abl in activated neutrophils, cells were fixed and permeabilized, and followed by staining c-Abl with H-300 and TRITC-conjugated secondary antibody and staining F-actin with FITC-conjugated phalloidin. All these stained cells were observed under the fluorescence microscope.

Kinase Assay—For activation, 1×10^7 neutrophils per sample were washed twice in H/S buffer and stimulated at 37°C with DREG56 (10 μg/ml). Cell stimulation was terminated by lysis in RIPA buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 2.5 mM sodium pyrophosphate, 1 mM each NaF, Na_3VO_4, and β-Glycerolphophate, and 20 μg/ml of aprotinin/leupeptin). After 15 min incubation on ice, cells were centrifuged at 15,000 × *g* for 15 min. Lysates were incubated with K12 (anti–c-Abl Ab). After 2 h, 40 μl of protein A sepharose beads (50% slurry) was added to the antibody/lysated mixture for 1 h. Immunoprecipitates were washed with lysis buffer (1 ml) at least four times, and then washed more than three times with the kinase buffer (25 mM Tris, pH 7.5, 2 mM DTT, 5 mM β-Glycerolphophate, 1 mM Na_3VO_4, 10 mM $MgCl_2$). After 5 min pre-incubation at 30°C, 30 μl of reactions were initiated by addition of 2 μg GST-CrkII-CTD and 5 μM ATP. 30 min later, reactions were terminated by addition of 20 μl 3× SDS sample buffer, and resolved by SDS-PAGE.

Immunoprecipitation and Immunoblotting—For the co-immunoprecipitation of c-Abl and L-selectin, neutrophils were stimulated and lysed as above. Lysates were centrifuged at 15,000 × *g* for 15 min, and the supernatants were incubated with K12 (anti–c-Abl antibody) or DREG56 (anti–L-selectin antibody). After 2 h, 40 μl of protein A/G sepharose beads (50% slurry) was added to the antibody/lysated mixture for 1 h. Immunoprecipitates were washed three to six times with lysis buffer and resolved by SDS-PAGE. Proteins were transferred to the nitrocellulose membranes by using chilled transfer buffer (25 mM Tris base, 192 mM glycerin, and 20% methanol) at 100 V for 1 h at 4°C. After protein transfer, nitrocellulose membranes were washed with TBST (20 mM Tris base, 500 mM NaCl, 0.05% Tween-20, pH 7.5) for more than three times and immediately incubated with 3% BSA and then indicated with primary antibody and HRP-conjugated secondary antibody at 37°C for 1 h. Chemiluminescent

detection was performed by using ECL plus Western-blotting reagents (Amersham biosciences).

RESULTS

1. L-Selectin Crosslinking Induces F-Actin Polymerization and Redistribution in Neutrophils—Activation of neutrophils by ligation of L-selectin is associated with a rapid cell shape change. We examined the F-actin formation after neutrophils were stimulated with L-selectin crosslinking. As shown in Fig. 1-I, Incubation with DREG56 alone could result in an increase in F-actin polymerization as measured by the fluorescence of FITC-phalloidin contrasted to the resting or control antibody treated neutrophils. F-actin assembly increased significantly if the neutrophils were crosslinked with DREG56 and its secondary antibody.

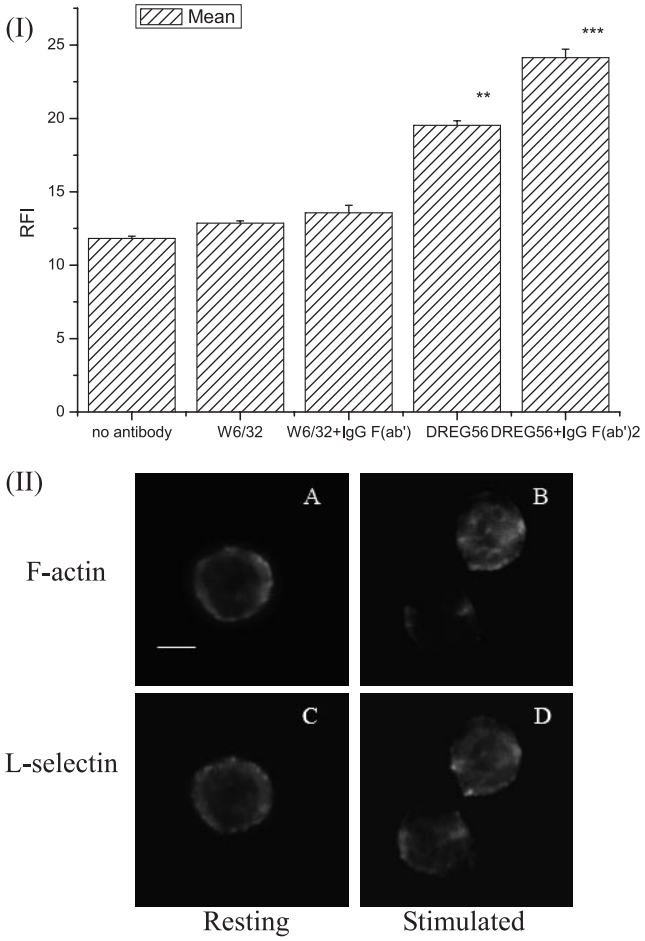

Fig. 1. **L-selectin crosslinking induces F-actin polymerization and redistribution in neutrophils.** I: Neutrophils were incubated with DREG56 alone or followed crosslinking with non-fluorescent secondary antibody. F-actin was stained by FITC-conjugated phalloidin. The amount of F-actin was quantified by flow cytometry. The values were expressed as relative fluorescence index (RFI). The significant difference of values was determined by One-way ANOVA (**$p < 0.01$, ***$p < 0.001$), and the data represent the mean ± SD from three independent experiments. II: The isolated neutrophils were incubated with DREG56 at 4°C for 30 min and TRITC-conjugated secondary antibody at 4°C for another 30 min. A part of cells were directly fixed, and another part of cells were incubated at 37°C for 10 min to lead L-selectin clustered, then the cells were fixed and stained for F-actin. A: F-actin in the resting neutrophils. B: F-actin in the activated neutrophils. C: L-selectin on the same resting cells. D: L-selectin on the same activated cells. Each image is representative of three independent experiments with cells from different donors. Bar = 20 μm.

We also investigated the redistribution of F-actin on the neutrophils activated by L-selectin crosslinking. The results showed that F-actin was faint and obscure in resting cells (Fig. 1-II, A). While in majority of stimulated neutrophils, F-actin was polarized and aggregated in several larger patches (Fig. 1-II, B). We observed that L-selectin was evenly distributed over the entire surface with some small patches or clusters in resting neutrophils (Fig. 1-II, C). In the activated cells, the patches of L-selectin localized with subjacent aggregates of F-actin (Fig. 1-II, D). These results demonstrated that L-selectin crosslinking can induce F-actin polymerization and polarization which is spatiotemporally related with the L-selectin alteration in the stimulated neutrophils.

2. Crosslinking of L-Selectin Increases c-Abl Kinase Activity and Induces c-Abl Kinase Localization to the Region Where F-Actin and L-Selectin Concentrated—Many studies have shown that ligation of L-selectin resulted in increased tyrosine phosphorylation of several cytosolic proteins. c-Abl is an important tyrosine kinase which is involved in the F-actin assembly in different cell lines. So c-Abl may be regulated when cells were stimulated by L-selectin ligation. We first investigated c-Abl kinase activity in the activated cells. To this end, stimulated neutrophils were lysed and immunoprecipitated, the anti–c-Abl immunoprecipitates were analyzed for kinase activity by using GST-CrkII-CTD as a substrate. As shown in Fig. 2-I, c-Abl kinase activity was elevated after L-selectin ligation contrasted to the control antibody treated cells. The increase of c-Abl kinase activity was more obvious if the neutrophils were crosslinked with L-selectin and its secondary antibody. By contrast, this increase in c-Abl kinase activity was greatly abrogated when the cells were incubated with STI571. These results suggested that c-Abl was regulated in the stimulated neutrophils triggered by L-selectin crosslinking.

We aslo focused on the localization of c-Abl in neutrophils before and after activation. The images showed that c-Abl and F-actin were approximatively uniform and evenly distributed within the resting cells (Fig. 2-II, A and B), while the majority of activated cells exhibited a polarized appearance with an extensive of c-Abl in the leading edge of the cells where F-actin polarized (Fig. 2-II, C and D). We next tested the relationships between the localization of c-Abl and the distribution of L-selectin before and after antibody crosslinking. The results showed that L-selectin and c-Abl were well-distributed and approximatively uniform within the resting cells (Fig. 2-III, A and B), whereas after activation caused by antibody crosslinking, c-Abl emitted more intensive fluorescence at the periphery of the cell where L-selectin polarized (Fig. 2-III, C and D). All these data suggested that L-selectin crosslinking can enhance c-Abl kinase activity and regulate c-Abl to the region where F-actin and L-selectin concentrated.

3. c-Abl Is Involved in the F-Actin Polymerization Caused by L-Selectin Crosslinking—To determine whether the nonreceptor tyrosine kinase c-Abl is involved in the

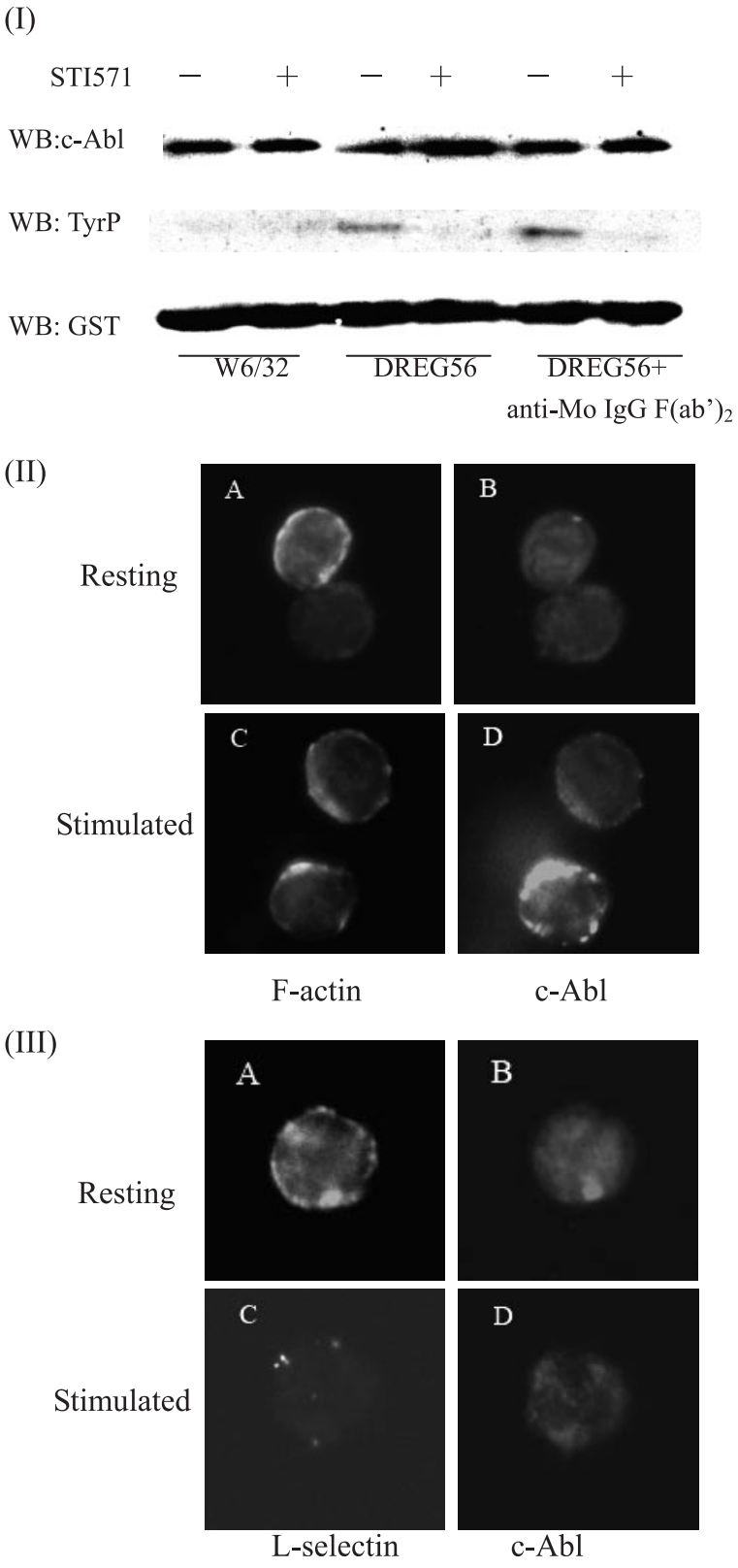

Fig. 2. **Crosslinking of L-selectin increases c-Abl kinase activity and induces c-Abl kinase localization to the region where F-actin and L-selectin concentrated.** I: 1×10^7 neutrophils per sample were washed twice in H/S buffer and stimulated at 37°C with DREG56 (10 μg/ml). Cell stimulation was terminated by lysis with the RIPA buffer. Lysates were incubated with K12 (anti–c-Abl antibody). After 2 h, 40 μl of protein A sepharose beads (50% slurry) was added to the antibody/lysated mixture for 1 h. The c-Abl immunoprecipitation was washed with the kinase buffer. After pre-incubation at 30°C for 5 min, 30 μl of reactions were initiated by addition of 2 μg GST-CrkII-CTD and 5 μM ATP. 30 min later, reactions were terminated and resolved by SDS-PAGE. Western blotting was conducted by using the mAb PY99, a specific for tyr(p), to test GST-CrkII-CTD. Chemiluminescent detection was performed by using ECL plus western-blotting reagents (Amersham biosciences). II: Neutrophils were crosslinked with DREG56 and non-conjugated secondary antibody. c-Abl was stained with H-300 and TRITC-conjugated secondary antibody, then F-actin was labeled with FITC-conjugated phalloidin. A: F-actin in the resting cells. B: c-Abl in the same resting neutrophils. C: F-actin in the antibody crosslinked cells. D: c-Abl in the same antibody crosslinked cells. III: Neutrophils were incubated with DREG56 at 4°C for 30 min and FITC-conjugated secondary antibody at 4°C for another 30 min. A part of cells were directly fixed, and another part of cells were incubated at 37°C for 10 min to lead L-selectin clustered, then the cells were fixed and stained for c-Abl. A: L-selectin on the resting neutrophils. B: c-Abl in the same resting neutrophils. C: L-selectin on the antibody ligated cells. D: c-Abl in the same antibody ligated cells.

F-actin assembly, neutrophils were treated with STI571 before L-selectin crosslinking. As shown in Fig. 3-I, a major decrease in the cellular F-actin content could be visualized, and the decrease was in a dose-dependent manner. Incubation of neutrophils with STI571 at concentrations up to 50 μM did not result in any increase in cell lysis or death in our experiments (not shown). The data obtained indicated that the alteration of F-actin–based

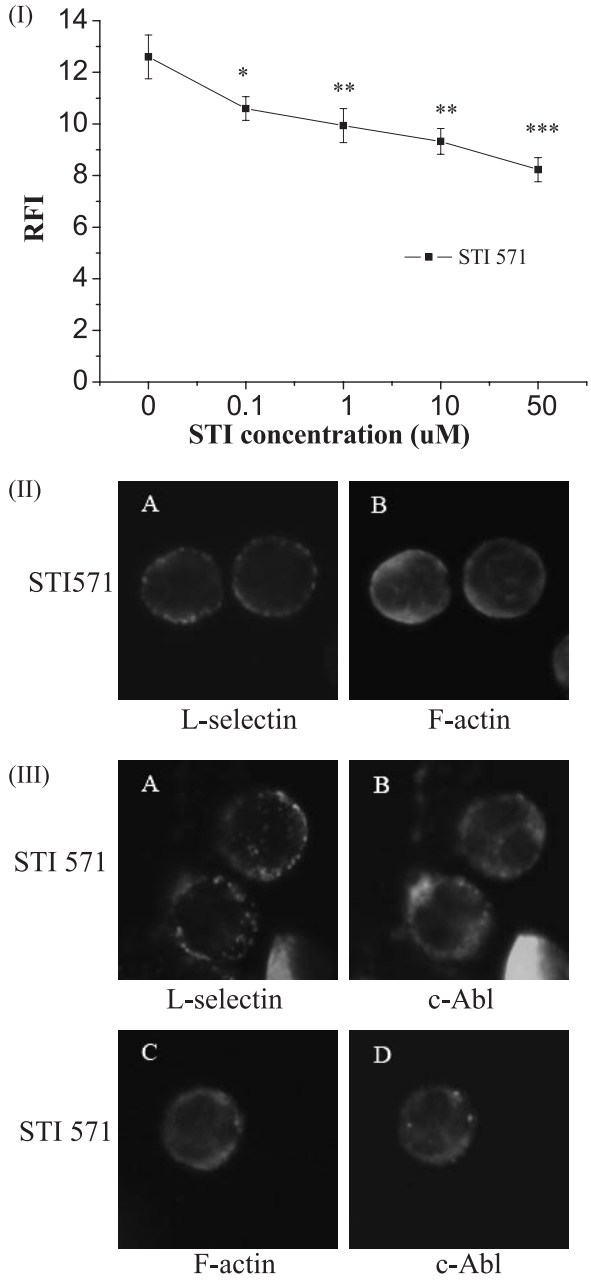

Fig. 3. **c-Abl is involved in the F-actin polymerization caused by L-selectin crosslinking.** I: Neutrophils were preincubated with STI571 at different concentrations (0.1 μM, 1 μM, 10 μM, 50 μM) at 4°C for 30 min and then cross-linked with DREG56. The group treated with equivalent volume of DMSO was considered as a positive control, and the values of inhibitor-treated groups were expressed as relative fluorescence index (RFI). Data represent the mean ± SD of three independent experiments and the significant difference from the control was determined by one-way ANOVA (*$p > 0.05$, **$p < 0.01$,***$p < 0.001$). II: Neutrophils were preincubated in the STI571 (10 μM) before crosslinking with DREG56. Cells were dually stained for L-selectin and F-actin. A: The distribution of L-selectin in the STI571 treated cells. B: The distribution of F-actin in the same STI571 treated cells. III: Neutrophils were preincubated with the STI571 (10 μM) before antibody crosslinking. Cells were dually stained for L-selectin and c-Abl, or for F-actin and c-Abl. A: L-selectin on the STI571 treated neutrophils. B: c-Abl in the same STI571 treated neutrophils. C: F-actin on the STI571 treated neutrophils. D: c-Abl in the same STI571 treated neutrophils.

cytoskeleton in neutrophils caused by antibody crosslinking was c-Abl involved.

To test if c-Abl was involved in the spatiotemporally co-localization of F-actin and L-selectin, neutrophils were pre-incubated with STI571 before cells were stimulated. Results showed that L-selectin polarization was reduced (Fig. 3-II, A), so did F-actin (Fig. 3-II, B). Incubation with STI571 also reduced the c-Abl intensive fluorescence and the spatiotemporally co-localization with L-selectin and F-actin (Fig. 3-III). All these results indicated that c-Abl was involved in the F-actin assembly triggered by L-selectin engagement.

4. L-Selectin, F-Actin and c-Abl Are Closely Related in the Activated Neutrophils—We have observed that c-Abl was regulated and centralized to the patches where F-actin and L-selectin concentrated following L-selectin crosslinking. It was of interest to identify the relationships between c-Abl, L-selectin and F-actin in the activated cells. Several reports have shown that the interactions between L-selectin and some cytoplasmic proteins may play an important role in F-actin assembly. To detect whether c-Abl was interacted with L-selectin after antibody ligation, cells were stimulated and then lysed for c-Abl/L-selectin immunoprecipitation. The immunoprecipitation complexes were resolved by SDS-PAGE and recognized with antibodies of DREG56/8E9. Results showed that c-Abl was associated with L-selectin when cells were stimulated with L-selectin engagement. Incubating with STI571 reduced the association between c-Abl and L-selectin (Fig. 4-I, A and B). In order to determine whether cytoskeleton is necessary for the connection of c-Abl and L-selectin, neutrophils were exposed to cytochalasin B at 100 μM of final concentration before antibody ligation. Results showed that cytochalasin B greatly reduced the connection of c-Abl and L-selectin (Fig. 4-II, A and B). Under a fluorescence microscope, we also observed that L-selectin clustering was largely abrogated (Fig. 4-III, A) and the polarization of c-Abl was severely inhibited (Fig. 4-III, B). All these data suggested that c-Abl, L-selectin and F-actin are closely related in the activated neutrophils caused by L-selectin engagement.

DISCUSSION

Neutrophil recruitment at sites of inflammation is regulated by a series of adhesion and activation events. Most published data suggested that selectins function as both adhesion molecules and transmembrane signaling receptors when leukocytes tethered and rolled on inflamed endothelium. Published data also suggested that L-selectin crosslinking induced rapid cell shape change that is correlated with F-actin alteration (*7*). In this study, we tested F-actin assembly in the L-selectin engagement or crosslinking neutrophils. Results showed that DREG56 incubation alone could induce F-actin assembly. The assembly of F-actin will be more significant when the cells were crosslined with DREG56 and its secondary antibody.

Reorganization of the actin cytoskeleton involves crosstalk between many adhesion molecules and some kinases that target the actin cytoskeletal machinery (*23, 24*). c-Abl is an important nonreceptor tyrosine kinase which is involved in the signaling transduction trigged by adhesion molecules. c-Abl contains a catalytic domain as well as a

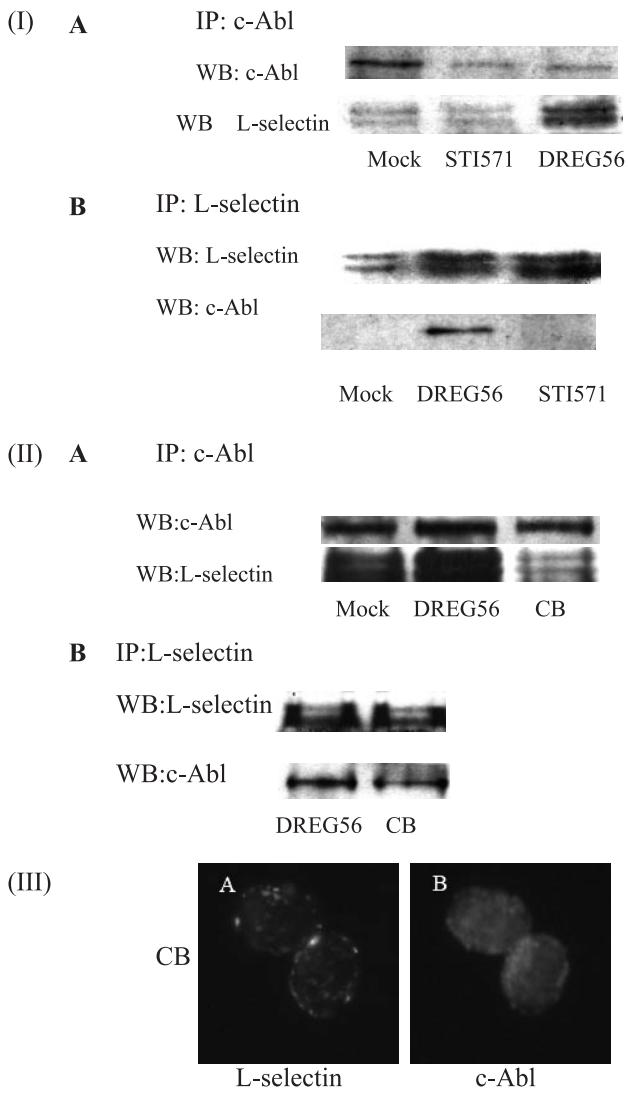

Fig. 4. **L-selectin, F-actin and c-Abl are closely related in the activated neutrophils.** I: Neutrophils were washed with H/S buffer and stimulated with DREG 56 (10 μg/ml) at 37°C for 5 min, then lysed with RIPA buffer. After 15 min incubation on ice, cells were centrifuged and the supernatants were incubated with the anti–c-Abl/anti–L-selectin antibody for immunoprecipitation. After 2 h, 40 μl of protein A/G sepharose beads (50% slurry) was added to the antibody/lysated mixture for 1 h. Immunoprecipitates were washed and resolved by SDS-PAGE. Proteins were detected with the DREG56/8E9. Chemiluminescent detection was performed by using ECL plus western-blotting reagents (Amersham biosciences). A: The c-Abl immunoprecipitation was resolved by SDS-PAGE and Western blotting was conducted with 8E9 and DREG56. B: The L-selectin immunoprecipitation was resolved by SDS-PAGE and Western blotting was conducted with DREG56 and 8E9. II: Neutrophils were preincubated in the cytochalasin B (100 μM) before crosslinking with DREG56, the c-Abl/L-selectin immunoprecipitation was resolved by SDS-PAGE and Western blotting was performed by DREG 56 and 8E9. A: The c-Abl immunoprecipitation was resolved by SDS-PAGE and western blotting was conducted with 8E9 and DREG56. B: The L-selectin immunoprecipitation was resolved by SDS-PAGE and western blotting was conducted with DREG56 and 8E9. III: Neutrophils were preincubated in the cytochalasin B (100 μM) before crosslinking with DREG56 and secondary antibody. Cells were dually stained for L-selectin and c-Abl. A: The distribution of L-selectin in the cytochasin B treated cells. B: The distribution of c-Abl in the same cytochasin B treated cells.

F-actin binding domain (*25–27*). Some extracellular signals that activate c-Abl also cause alterations in the F-actin cytoskeleton. In our study, we observed that the cytoplasmic c-Abl localized to the dynamic regions of cytoskeleton, including the leading edges and F-actin protrusions of the activated neutrophils caused by L-selectin crosslinking. Inhibition of c-Abl kinase activity with STI571 and the specific inhibitor of actin polymerization cytochalasin B could reduce the quantification of F-actin fluorescence and inhibit c-Abl polarizing to the leading edges of the cells where F-actin concentrated.

Actually, F-actin can inhibit c-Abl activity in vitro by direct interaction with the F-actin–binding motif at the extreme C-terminus of c-Abl (*27*). In our present work, we tested the c-Abl kinase activity in the activated neutrophils. The data showed that c-Abl kinase activity was greatly increased contrasted to the control antibody treated cells. The increase of c-Abl kinase activity was greatly reduced by incubating with STI571 before neutrophils were stimulated. The increased c-Abl kinase activity was correlated with the increasing quantities of F-actin fluorescence. So we supposed that the positive effect of c-Abl on the F-actin alteration triggered by L-selectin crosslinking might be balanced by the negative effect of F-actin on c-Abl kinase activity to limit the extent F-actin formation.

Antibody crosslinking, hyperthermic treatment and ligand binding studies demonstrated that the cytoplasmic tail of L-selectin is important to the function of L-selectin (*28, 29*). In our experiments, we found that c-Abl reclined to the patches where L-selectin clustered. Inhibition of c-Abl kinase activity with STI571 can inhibit c-Abl and L-selectin spatially co-localization at the leading edges of the cells. Furthermore, we found that c-Abl and L-selectin are co-immunoprecipitated in neutrophils. STI571 and cytochalasin B could reduce the association between c-Abl and L-selectin. All these results together showed that c-Abl tyrosine kinase may be interacted with L-selectin in an F-actin dependent manner when neutrophils were stimulated by L-selectin ligation.

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