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p85-RhoGDI2, a novel complex, is required for PSGL-1-induced β1 integrin-mediated lymphocyte adhesion to VCAM-1

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ABSTRACT

P-selectin glycoprotein ligand-1 and β1 integrin play essential roles in T cell trafficking during inflammation. E-selectin and vascular cell adhesion molecule-1 are their ligands expressed on inflammation-activated endothelium. During the tethering and rolling of lymphocytes on endothelium, P-selectin glycoprotein ligand-1 binds E-selectin and induces signals. Subsequently, β1 integrin is activated and mediates stable adhesion. However, the intracellular signal pathways from PSGL-1 to β1 integrin have not yet been fully understood. Here, we find that p85, a regulatory subunit of phosphoinositide 3-kinase, forms a novel complex with Rho-GDP dissociation inhibitor-2, a lymphocyte-specific RhoGTPases dissociation inhibitor. Phosporylations of the p85-bound Rho-GDP dissociation inhibitor-2 on 130 and 153 tyrosine residues by c-Abl and Src were required for the complex to be recruited to P-selectin glycoprotein ligand-1 and thereby regulate β1 integrin-mediated T cell adhesion to vascular cell adhesion molecule-1. Both shRNAs to Rho-GDP dissociation inhibitor-2 and p85 and over-expression of Rho-GDP dissociation inhibitor-2 Y130F and Y153F significantly reduced the above-mentioned adhesion. Although Rho-GDP dissociation inhibitor-2 in the p85-Rho-GDP dissociation inhibitor-2 complex was also phosphorylated on 24 tyrosine residue by Syk, the phosphorylation is not required for the adhesion. Taken together, we find that specific phosphorylations on 130 and 153 tyrosine residues of p85-bound Rho-GDP dissociation inhibitor-2 are pivotal for P-selectin glycoprotein ligand-1-induced β1 integrin-mediated lymphocyte adhesion to vascular cell adhesion molecule-1. This will shed new light on the mechanisms that connect leukocyte initial rolling with subsequent adhesion.

1. Introduction

It has been well established that inflammation is initiated by leukocytes rolling on and subsequently attaching to the activated endothelium, and followed by inflammatory cells' trans-endothelium migration toward the infected or damaged sites (Zarbock et al., 2009). P-selectin glycoprotein ligand-1 (PSGL-1), the well-known ligand for P-, E-, and L-selectins (Norman et al., 2000; Sperandio et al., 2003; Carlow et al., 2009), is constitutively expressed on the tips of leukocyte microvilli (von Andrian and M'Rini, 1998). During inflammation, P- and E-selectins are expressed on the activated endothelium (Sundd et al., 2012; Buffone et al., 2013). PSGL-1 binding to P- or E-selectin mediates leukocyte rolling on endothelium, which is the initial step of leukocyte migration (Zarbock et al., 2009). Subsequently, integrins are activated and mediate the arrest and stable adhesion of leukocytes to endothelium (Lefort and Ley, 2012).

The importance of PSGL-1 has emerged beyond a mechanical anchor as a selectin ligand. A growing list of kinases such as MAPK (Hidari et al., 1997), Syk (Abbal et al., 2006; Spertini et al., 2012), c-Abl (Ba et al., 2005a,b) and Src (Xu et al., 2007) has been found activated by PSGL-1 engagement. It has been reported that PSGL-1/selectin binding triggers signal transduction to activate αLβ2 integrin in a gradual manner (Abbal et al., 2006; Alon and Ley, 2008; Simon et al., 2000). Beside leukocyte-specific β2 integrin, ubiquitously expressed β1 integrins constitute another

Abbreviations: E-Fc, recombinant human E-Selectin/CD62E Fc chimera; RhoGDI2, Rho-GDP dissociation inhibitor-2; STI, STI571; Pic, Piceatannol; PI3K, phosphoinositide 3-kinase; PSGL-1, P-selectin glycoprotein ligand-1; VCAM-1, vascular cell adhesion molecule-1; WCL, whole cell lysate.

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major membrane protein on lymphocytes. CD2 or CD47 triggering induced the adhesion of Jurkat lymphocytes to VCAM-1 expressed on the activated endothelium via β1 integrin (Rosenthal-Allieri et al., 2005). Whether lymphocyte PSGL-1-induced signals result in β1 integrin-mediated adhesion is worthy of investigation to understand the corporation of adhesion molecules during the initial rolling and subsequent stable adhesion in lymphocyte migration, which accounts for the pathogenesis of T cell-involved inflammatory diseases.

Class IA phosphoinositide 3-kinase (PI3K) is a critical lipid kinase in T cell receptor- and B cell receptor-triggered signals (Vogel and Fujita, 1993; Shan et al., 2000; Dai et al., 2006). p85, the regulatory subunit of PI3K, has been paid particular attention in PI3K signaling pathways (Cantrell, 2001). Besides, p85 also functions as an adaptor protein by binding to other signal molecules, including IRS-1 (Sun et al., 1991), Grb2, Gab1/2 (Sattler et al., 2002), Shc (Harrison-Findik et al., 1995; Gu et al., 2000), Crk-L (Sattler et al., 1996), β-catenin (Woodfield et al., 2001), Rac (Reynolds et al., 2002), Lck and Fyn (Prasad et al., 1993; Pleiman et al., 1994). Twenty years of investigation has yielded a detailed view of the regulatory role of p85 for PI3K activity (Cantrell, 2001); however, less progress has been made in p85 as an adaptor protein since 2002 (Mellor et al., 2012).

In PSGL-1 signaling pathways, PI3K is activated by recruiting the p85 subunit to PSGL-1 cytoplasmic domain, and thereby activates β2 integrin (Wang et al., 2007). Our previous work also identified PI3K's pivotal role in PSGL-1-mediated neutrophil rolling on E-selectin (Luo et al., 2010), suggesting the involvement of p85 in PSGL-1 signal transduction. Interestingly, we found a novel p85-Rho-GDP dissociation inhibitor-2 (RhoGDI2) complex that functions independently of PI3K in the present study. We further investigated its crucial role in PSGL-1-induced β1 integrin-mediated lymphocyte adhesion to VCAM-1. To our knowledge, this is the first report of the p85-RhoGDI2 complex in PSGL-1 signaling to β1 integrin.

2. Materials and methods

2.1. Reagents and antibodies

KPL1 (anti-PSGL-1 monoclonal antibody, mouse IgG1, SC-13535), and antibodies to Src (H12, SC-5266), Syk (SC-1240), c-Abl (K12, SC-131) and p85 (SC-1637) were purchased from Santa Cruz Biotechnology. Rabbit antiserum against p85 (06-195) was from Upstate. Anti-RhoGDI2 rabbit antiserum (D9937), PY20 (anti-phosphotyrosine monoclonal antibody), Piceatannol (Syk/ZAP-70 specific inhibitor) and LY294002 (PI3K inhibitor) were from Sigma–Aldrich. STI571 (specific inhibitor to c-Abl) was a gift of Novartis Pharma Schweiz AG (Basel, Switzerland). PP2 (Src family kinase-specific inhibitor) was from Calbiochem-Novabiochem (San Diego, CA, USA). Glutathione (GSH)-sepharose 4BTM (17-0756-01) was from Amersham Biosciences (Piscataway, NJ, USA). Mouse IgG (12-371), CBL481 (a mouse blocking antibody to β1 integrin), and anti-PI3 Kinase p110α (09-481) were from Millipore. VCAM-1 and E-Fc (recombinant human E-Selectin/CD62E Fc chimera) were from R&D. Phosphorylation-specific antibodies to c-Abl-412-TyrP, Syk-525/526-TyrP and Src-416-TyrP were from cell signaling.

Table 1
Primers for indicated plasmids.

Plasmids	Sequence
GST-p85 p1BCRp2	5′CCGGATCCTCGCCTCCCACACCAAAGC3′ 5′CCCTCGAGCTAAGGTTTTGGTGGTTTAGGA3′
His-RhoGDI2	5′CCGGATCCATGACTGAAAAAGCCCCAGAGCC3′ 5′CCCTCGAGTCATTCTGTCCACTCCTTC3′
GFP-RhoGDI2	5′CGACGCGTCCTTCTGTCCACTCCTTCTT3′ 5′CGGGATCCATGACTGAAAAAGCCCCA3′

2.2. Cell culture

Jurkat T cells (Clone E 6.1) and 293T from ATCC were maintained in medium supplemented with 10% FBS, 100 U penicillin, and 100 μg/mL streptomycin.

2.3. Preparation of human T lymphocytes

Batches of blood (25 mL) from donors at the Blood Center of Changchun were layered on top of 20 mL Ficoll 1.007 density gradient and centrifuged at 400 × *g* for 20 min at room temperature. Then the peripheral blood mononuclear cell layer was collected, washed twice in DMEM and resuspended at 10–12 × 10^6 cells/mL in DMEM supplemented with 10% FBS and then isolated by CD3 MicroBeads (130-050-101, Miltenyi Biotec) according to the manufactures' protocol.

2.4. Plasmids cloning

pGEX plasmids encoding full-length p85, the C-terminal SH2 domain (C-SH2), and the N-terminal SH2 domain (N-SH2) as GST fusion proteins were gifts from Lucia E. Rameh (Boston Biomedical Research Institute, USA) and Fabrice Gouilleux (Université François Rabelais de Tours, Parc de Grandmont, France). p85-p1-BCR-p2 was amplified from full-length p85 and inserted into pGEX-6p-1 using primers shown in Table 1. RhoGDI2 was subcloned into the pET-28a(+) after amplification from Jurkat cells using indicated PCR primers (Table 1). Mutations of putative phosphorylation sites in RhoGDI2 (Y24F, Y130F and Y153F) were introduced by Easy Mutagenesis System (FM101, TransGen Biotech). Sequences encoding RhoGDI2 WT, 130F and 153F were inserted into the lentiviral pWPXLd (Invitrogen) vector by BamHI and MluI to get GFP-RhoGDI2 WT, 130F and 153F (Table 1).

For shRNA preparation, annealed double-stranded shRNA oligonucleotides shown in Table 2 were cloned into the BamHI and

Table 2
Oligonucleotides used for generating shRNA.

Targeted regions	Sequence
Control	5′GATCCCCTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCGGAGAATTTTTC3′ 5′TCGAGAAAAATTCTCCGAACGTGTCACGTTCTCTTGAAACGTGACACGTTCGGAGAAGGG3′
RhoGDI2Sh1 (306-324)	5′GATCCCCGGAAGGTTCTGAATATAGATTCAAGAGATCTATATTCAGAACCTTCCTTTTTC3′ 5′TCGAGAAAAAGGAAGGTTCTGAATATAGATCTCTTGAATCTATATTCAGAACCTTCCGGG3′
RhoGDI2Sh2 (373-391)	5′GATCCCCTACGTTCAGCACACCTACATTCAAGAGATGTAGGTGTGCTGAACGTATTTTTC3′ 5′TCGAGAAAAATACGTTCAGCACACCTACATCTCTTGAATGTAGGTGTGCTGAACGTAGGG3′
p85Sh1 (209-227)	5′GATCCCCCGAAGAATATATTCAGCTATTCAAGAGATAGCTGAATATATTCTTCGTTTTTC3′ 5′TCGAGAAAAACGAAGAATATATTCAGCTATCTCTTGAATAGCTGAATATATTCTTCGGGG3′
p85Sh2 (648-666)	5′GATCCCCCGGGAACTTACGTAGAATATTCAAGAGATATTCTACGTAAGTTCCCGTTTTTC3′ 5′TCGAGAAAAACGGGAACTTACGTAGAATATCTCTTGAATATTCTACGTAAGTTCCCGGGG3′