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Western Blot Analysis of Adhesive Interactions under Fluid Shear Conditions: The Blot Rolling Assay

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Summary

Western blotting has proven to be an important technique in analysis of receptor-ligand interactions (i.e., by ligand blotting) and for identifying molecules mediating cell attachment (i.e., by cell blotting). Conventional ligand blotting and cell blotting methods employ nondynamic (static) incubation conditions, whereby molecules or cells of interest are placed in suspension and overlaid on membranes. However, many cell–cell and cell–matrix adhesive interactions occur under fluid shear conditions, and shear stress itself mediates and/or facilitates the engagement of these physiologically appropriate receptors and ligands. Notably, shear forces critically influence the adhesion of circulating cells and platelets to vessel walls in physiologic cell migration and hemostasis, as well as in inflammatory and thrombotic disorders, cancer metastasis, and atherosclerosis. Use of nondynamic blotting conditions to analyze such interactions can introduce bias, overtly missing relevant effectors and/or exaggerating the relative role(s) of nonphysiologic adhesion molecules. To address this shortfall, we have developed a new technique for identifying binding interactions under fluid shear conditions, the “blot rolling assay.” Using this method, molecules in a complex mixture are resolved by gel electrophoresis, transferred to a membrane that is rendered semi-transparent, and the membrane is then incorporated into a parallel-plate flow chamber apparatus. Under controlled flow conditions, cells or particles bearing adhesion proteins of interest are then introduced into the chamber and interactions with individual immobilized molecules (bands) can be visualized in real-time. The substrate molecule(s) supporting adhesion under fluid shear can then be identified by staining with specific antibodies or by excising the relevant band(s) and performing mass spectrometry or microsequencing of the isolated material. This method thus allows for the identification, within a complex mixture and without prior isolation or purification, of both known and previously uncharacterized adhesion molecules operational under dynamic conditions.

Keywords

Adhesion molecules; Shear stress; Shear conditions; Fluid shear; Flow conditions; Parallel plate flow chamber; Western blot; Selectins; Selectin ligands; Cell blotting; Blot rolling assay; Gel electrophoresis

1. Introduction

Adhesive interactions of cells with other cells and/or extracellular matrix under fluid shear conditions are critical to a variety of physiologic and pathobiologic processes, including

hemostasis, leukocyte trafficking, tumor metastasis, and atherosclerosis. Although various techniques for analyzing cell adhesion have been described, most involve binding assays under nondynamic (static) conditions. Importantly, certain adhesive receptor–ligand interactions occur preferentially if not solely under physiologic shear stress (e.g., binding of L-selectin to its ligands) or depend on low affinity and rapidly reversible interactions to serve their functions (e.g., rolling of leukocytes via selectins). Under static binding assay conditions, effectors mediating these types of interactions may be overtly neglected or overshadowed by molecules specialized to form more stable adhesions.

Methods for in vitro study of adhesion under dynamic conditions have been described, including the Stamper–Woodruff (1) and the parallel plate flow chamber (2) assays. However, these methods are limited in that they require the availability of reagents (e.g., antibodies) that can specifically interfere with the activity of relevant receptors and/or ligands or require isolated substrate materials that can be immobilized on the chamber surface. Thus, the applicability of these methods to examine the structure or function of previously unrecognized/uncharacterized ligands, especially within a complex mixture, is highly constrained.

To address these issues, we developed a method for direct real-time observation of adhesive interactions between cells or particles in flow and proteins separated by SDS-PAGE and immobilized on membranes (3). This technique, which we have termed the “blot rolling assay,” has allowed for the identification of new glycoprotein ligands (4–6), as well as a glycolipid ligand (7) for selectins. This method allows real-time assessment and measurement of interaction parameters (e.g., rolling vs. firm attachment, specificity, and reversibility with inhibitors) in both physiologic and nonphysiologic shear conditions, thus permitting a unique user interface for the observation of adhesive events on membrane-immobilized materials. Ligands under investigation can be immobilized directly or segregated by gel electrophoresis (e.g., SDS-PAGE, isoelectric focusing) or other methods prior to transfer to the membrane. This method also provides for real-time manipulation of interaction conditions including wall shear stress, ion requirements, temperature, the influence of metabolic inhibitors, and the presence of activating agents or inhibitors of cell function. Blot-immobilized substrates can be used repeatedly, allowing in situ manipulation of the substrate under continuous direct visualization or direct comparison of different conditions or different cell populations in shear flow. The capacity to observe sequential experimental and control conditions on a single substrate, and to observe physiologic behaviors and responses to manipulations in real-time, provides particularly powerful advantages of this method over conventional static binding assays. Similarly, the ability to observe interaction with individual components of a complex mixture without requiring prior purification or knowledge of the nature of the components provides advantages over conventional flow-based assays. In this chapter, we will describe a detailed protocol for performing blot rolling assays of membrane proteins. A video recording of a representative experiment will be presented to illustrate the utility of this new analytic technique.

2. Materials

The required components for performing blot rolling assays include an inverted microscope and a parallel plate flow chamber apparatus. Figure 1 shows the setup utilized in our laboratories. The parallel plate device we use has internal chamber diameters of $2\text{ cm} \times 0.5\text{ cm} \times 0.025\text{ cm}$. A commercial product with similar properties is available from GlycoTech Corp., Rockville, MD, USA (Circular flow chamber, Product #31–001). Flow is adjusted by negative pressure regulated by a high precision, programmable syringe pump (Harvard Apparatus). Wall shear stress (dyne/cm^2) is calculated according to the formula: Wall Shear Stress (τ) = $6\mu Q/wh^2$, where w is the chamber width in cm, h the chamber height in cm, Q the volumetric flow rate in mL/s, and μ is the viscosity in poise, or $\text{dyne} \times \text{s}/\text{cm}^2$; for water at 25°C , $\mu = 0.009$, which is adjusted for contribution of 10% glycerol (*see below*), according to the equation $\mu = 1.37 \times \text{medium}$ ($\mu = 0.0123$ at 25°C) (*see below* for calculations based on our chamber dimensions). Interaction events can be captured on videotape by using a standard CCD camera and video recording assembly (*see Fig. 1*), for later off-line analysis.

1. PAGE gel system.
2. equi-Blot™ PVDF Membrane (Bio-Rad, Hercules, CA, USA, #162–0184).
3. Fetal bovine serum.
4. Phosphate buffered saline/0.1% Tween-20 (PBST).
5. Prestained molecular weight markers.
6. Western Blue stabilized substrate for alkaline phosphatase (Promega #S3841).
7. Blot rolling medium (H/H Medium): Hank's Balanced Salt Solution (Gibco #14170–112) with 10 mM HEPES, pH 7.4 (Cellgro #25–060). For “binding medium”, add 2 mM CaCl_2 and 10% glycerol to H/H medium.
8. Monoclonal or polyclonal antibody reagents for identifying any epitope(s) of interest (*see Note 1*).
9. Acrylamide gradient gels (Criterion 4–20%; Bio-Rad).
10. 1% *n*-octylglucoside (Roche Molecular Biochemicals, Indianapolis, IN, USA).

3. Methods

3.1. Preparation of Western Blots/Visualization of Protein Bands

1. Material of interest can be prepared for PAGE (with or without SDS) using standard methods. In general, we prepare lysates of cells at 4×10^8 cell equivalents per mL of lysate buffer. A number of standard detergents have been utilized for preparation of lysates, though we have found 1% *n*-octylglucoside to provide low nonspecific background binding. Acrylamide gradient gels provide

¹We typically avoid immunoblotting with function blocking antibodies, since these antibodies may interfere with cell binding to the membrane (*see Subheading 3.4, Step 7*).

improved resolution in higher molecular weight ranges. The amount of protein required will vary with the material under study, but we have found 25–100 µg of protein per lane to be sufficient for most of our studies. Both reducing and nonreducing gel conditions have been utilized with good results. Omission of detergent from the gel and/or the nondenaturing gel electrophoresis systems can be utilized to optimize structural integrity of resolved bands. In all gels, it is helpful to include prestained molecular weight standards in the lanes adjacent to the material of interest to assist in alignment and localization of regions supporting adherence (*see* Notes 2 and 3).

2. When gel electrophoresis is complete, the component proteins are transferred to PVDF membrane using standard transfer methods; although other membrane materials may work as well, we have preferred BioRad Sequi-Blot™, which has 0.2/µm pore size and minimal surface irregularities, thus reducing turbulence and promoting laminar flow over the membrane (*see* Note 4).
3. After transfer, the blot is placed protein side facing up (i.e., the side of the membrane that was facing the gel) in a blocking solution (50% FBS in PBST and 100% newborn calf serum have been used with good results) to reduce nonspecific interactions and incubated with gentle agitation (on a rocker platform or rotating platform) for 1 h at room temperature. The blot is then washed twice in PBST for 5 min each with gentle agitation.
4. Immunostaining, if desired, can be performed using standard procedures. Membranes are typically stained with primary antibody for 1 h under gentle agitation, washed 2× in PBS/0.1% Tween 20 for 5 min and then incubated with secondary alkaline phosphatase-conjugated antibody in TBS (Tris buffered saline) for 1 h under gentle agitation. The blot is again washed 2× in PBST for 5 min, then rinsed in PBS followed by TBS (to remove residual PBST), and then developed with alkaline phosphatase substrate (e.g., Western Blue™). After developing blots to an appropriate signal-to-background perspective, reactions are stopped with PBS and the blot is washed twice with PBS. Blots to be used for flow experiments may be stored in buffer at 4°C until use. Blots will maintain binding fidelity for several days, though best results are obtained with freshly prepared materials (*see* Note 5).

²Any uniform percent gel or gradient gel can be used so long as sufficient resolution of protein bands is achieved in the molecular weight range of interest.

³If one wishes to measure the relative strength of interactions between component bands of samples loaded in different lanes of the same gel, it is important to load samples in lanes an appropriate distance apart such that the membrane-attached flow chamber does not cover another sample lane in order to avoid damaging the latter. For our flow chamber and the Criterion precast gel system, we load samples in every third lane, using molecular weight markers in between.

⁴Surface irregularities can result in disruption of cell interactions on the membrane. Some membrane surface irregularities due to manufacture are readily visible when the membrane is immersed in methanol prior to the transfer step, while others are only observable when the membrane is immersed in 10% glycerol. Avoid the use of visibly scored or scratched membranes, as these defects may prevent a proper vacuum seal of the chamber to the membrane or provide a “trough” in which perfused cells may collect and subsequently adhere nonspecifically.

⁵In all incubations with mAb or serum, it is critical to avoid using bacterially contaminated solutions in order to prevent digestion of binding epitopes by bacterial proteases and/or glycosidases.

5. The membranes are again blocked by incubation in 50% newborn calf serum or FBS in binding media (H/H with Ca^{2+} /10% glycerol) for at least 1 h at 4°C and equilibrated in binding media prior to use in the flow assay.

3.2. Preparation of Selectin-Expressing Cells

To assay blots for selectin ligand activity, we typically employ lymphocytes expressing L-selectin or Chinese hamster ovary (CHO) cells stably transfected to express P-selectin or E-selectin.

1. Human peripheral blood lymphocytes (PBL) can be prepared from whole blood by Ficoll density gradient separation. For analysis of L-selectin function on lymphocytes, monocytes may be removed by plastic adherence or other methods to reduce nonspecific binding (e.g., magnetic bead separation). Murine spleen and lymph node T cells and rat thoracic duct lymphocytes have also been utilized in the same fashion. E-selectin and P-selectin transfected cell lines are typically split the day prior to use to achieve log phase growth and are harvested by replacing the media with H/H 5 mM EDTA. The cells can then be released from the flask by manual agitation or scraping. We do not use trypsin to release the cells as this may alter the function of some surface adhesion molecules.
2. After counting, the cells are suspended at 10× the concentration desired for use in the flow chamber (e.g., $10\text{--}20 \times 10^6/\text{mL}$ in H/H without Ca^{2+} or Mg^{2+} and without glycerol) and stored on ice. Clumping of adherent cell populations (i.e., CHO cells) may develop after prolonged storage on ice. This can be remedied by washing again in H/H 5 mM EDTA before use (*see* ^{Note 6}).
3. Controls to confirm specificity for selectin binding to blots include use of mock-transfected CHO cells and/or preincubation of cells with function-blocking anti-selectin mAb.

3.3. Inverted Microscope Setup

1. Setup will vary with type of microscope. In general, we do not use any filters.
2. Figure 2 shows the placement of the parallel plate chamber on the blot. Begin visualization at low power to align the chamber and identify the area of interest. Many observations are best performed at relatively low magnification (e.g., 10× objective) to allow observation of larger areas and numbers of cells. Observation under increased magnification can be performed to observe individual cell characteristics. Adjust the shutter arm so that the light narrows specifically over the area of interest on the blot.

3.4. Blot Rolling Assay

1. All media should contain 10% glycerol. Equilibration in dilute glycerol alters the opacity of the PVDF membrane sufficiently to allow transmission of light and

⁶It is important that cells are not stored in glycerol, as a 10% glycerol solution will significantly decrease cell (e.g., CHO) viability within 15 min.

the direct visualization of cells interacting with the surface of the blot by standard light microscopy. Assessment of some functions (e.g., integrin activation) requires maintaining the chamber and media at 37°C. This can be achieved with a stage warming device and placement of supply media in water baths adjacent to the apparatus (*see*^{Note 7}).

2. The chamber should be mounted onto the blot so that the flow channel is aligned over the lane of interest. The flow chamber is secured by application of low vacuum pressure in the same fashion as is used for attachment to glass or plastic surfaces. We have not found it necessary to perform any special maneuvers to maintain an adequate seal on Sequi-Blot PVDF membrane (*see*^{Note 8}).
3. The flow field is rinsed with media introduced through the 20-mL syringe, attached to the three-way stopcock, to remove bubbles and prepare the blot surface for cell input (*see*^{Note 9}).
4. Immediately before use, aliquots of cells (e.g., PBL or transfected CHO cells) are diluted tenfold into binding medium (H/H w/Ca²⁺ with 10% glycerol; final concentration is typically $1\text{--}2 \times 10^6$ cells/mL) at room temperature (or 37°C as indicated). This minimizes exposure to glycerol and divalent cations that may affect cell viability and/or promote the formation of cell aggregates (*see*^{Note 10}).
5. The cell input line is flushed with assay medium and placed into the tube containing cells. Flow is regulated by function of a downstream syringe pump. Use of a programmable pump allows for automation of the assay technique and more reproducible results. We typically initiate flow at a high rate of flow (2 mL/min or ~ 7.5 dyne/cm²) to bring cells through the sample tubing and into the chamber. Upon arrival of cells in the field of view, the flow rate is reduced (typically to 0.5–1 dyne/cm²) to allow cells to interact with the blot surface. The flow rate can be adjusted up or down to increase or decrease, respectively, the stringency of binding interactions. One can program step-wise increases in flow (shear stress) without interruption. Maintaining continuous flow while cells are in the chamber minimizes nonspecific binding to the blot surface. This is critical with adherent cell populations such as CHO cells or monocytes, though lymphocytes typically do not bind when flow is stopped for brief periods (<30 s).
6. Observation of cell interactions with substrate molecule(s) are made in real time and recorded via the camera/VCR for later analysis. Typically, two types of analyses are performed. First, a “scanning” analysis is performed by moving the microscope stage to view the entire length of the lane where proteins have been

⁷Viscosity is temperature dependent. If the assay temperature is adjusted from 25 to 37°C, make sure to recalculate flow rates using the appropriate viscosity values for cell perfusion at specific shear stresses (*see* Subheadings 2 and 3).

⁸We use Dow Corning High-Vacuum Grease to seal the silicon rubber gasket to the flow chamber to create the flow channel. In order to prevent the gasket from slipping after placement, we allow the grease to set for 24 h before use in experiments. It is critical to remove all excess vacuum grease from the channel, gasket surface, and other flow chamber surfaces completely (using a cotton swab and 70% ethanol) before use in blot rolling assays to prevent the transfer of grease onto the membrane, which will damage the lanes and render them unusable.

⁹All bubbles and debris from the channel must be flushed to clear all potential for flow disturbances.

¹⁰The cell concentration can be adjusted to increase or decrease assay sensitivity as desired (i.e., higher cell concentration increases sensitivity).

resolved while maintaining a constant shear rate. Tethering and rolling interactions are usually most prominent at the leading edge of a “band” on the blot, with rolling cells evident across the band and discontinuation of rolling interactions observed at the downstream edge. Once bands or areas of the blot of interest are identified, we typically perform additional studies comparing control cell preparations (mock-transfected or cells incubated with function blocking antibodies) and varying shear conditions while viewing a fixed area or band on the blot. In this analysis, tethering is observed at low physiologic wall shear stresses (0.5–1.5 dyne/cm²) for short periods (one to several minutes). Bound cells are then subjected to timed stepwise increases in wall shear stress to assess shear resistance as an estimate of relative strength of binding. The dependence of interactions on the presence of calcium (typical of selectin-mediated binding) can be confirmed by perfusion of the chamber with H/H with 10% glycerol and 5 mM EDTA and observing release of bound cells, or by repeating the analysis using H/H with 10% glycerol and 5 mM EDTA for both dilution of the cells and perfusion through the chamber.

7. Antibody inhibition experiments utilizing antibodies to the membrane bound ligand can be performed in situ by first observing interactions on an area of defined interest, then perfusing the chamber with a solution of antibody (e.g., 1–10 µg/mL for 30 min) and repeating the observation on the same site. If the antibody blocks binding interactions, then the blot is unusable for further studies of that ligand. However, if the applied antibody does not block adhesive interactions, the blot may be reused, i.e., successive rounds of antibodies or reagents may be screened in this fashion.
8. For selectin-mediated binding, cells must tether and roll in shear flow to be considered specific. Tethering in our studies is defined as reduction of forward motion below the hydrodynamic velocity lasting a minimum of two video frames (0.07 s), and rolling is defined as >5 cell diameters of lateral translation below the hydrodynamic velocity. The majority of tethered cells in such studies are observed to roll smoothly across the entire band or field of view. Nonspecific interactions (i.e., cellular collisions with the substrate that did not lead to tethering and/or rolling) are defined as interactions lasting less than 0.07 s and are not included in the analysis. Nonspecifically bound cells (not rolling and/or not released by perfusion with EDTA) are also discounted from analysis. In general, there is a time-dependent increase in nonspecific (nonrolling) attachments in the absence of flow, though it is our experience that very few cells form nonspecific attachments to the blot in continuous shear flow. Tethering rate is calculated as the number of cells that tether per field per time at a defined shear stress and adjusted to per minute values. As stated above, wall shear stress (τ) values are calculated according to the formula τ (dynes/cm²) = $6\mu Q/wh^2$ where μ is the viscosity of the solution in the chamber in poise, Q is the volumetric flow rate in mL/s, w is the channel width (e.g., 0.5 cm for chamber we employ), and h is the channel height (e.g., 0.0127 cm for chamber we employ) (8). A value of 0.009 poise is used for the viscosity (μ) of water at 25°C

(9) and a value of 0.0123 poise is used for the viscosity of 10% glycerol (v/v) in water at 25°C (10). By these values, wall shear stress in 10% glycerol at 25°C is approximately 1.37-fold greater than in water at the same temperature.

9. Regions (bands) of interest can be aligned with molecular weight markers run in adjacent lanes to estimate molecular weight. Additionally, one may subsequently probe the blot with antibody to specific proteins of interest to determine whether band(s) supporting binding represent known proteins. This approach has been used to confirm PSGL-1 and identify CD43 as selectin ligands (3, 6). One can also excise regions of blot supporting binding and submit for mass spectrometry and/or protein microsequencing. This approach was used to identify HCELL, a glycoform of CD44 that functions as a high affinity L-selectin and E-selectin ligand (4, 5). Figure 3 is a representative histogram of a blot rolling assay of T cell lysates probed with stably transfected Chinese hamster ovary (CHO) cells expressing P-selectin (CHO-P) and E-selectin (CHO-E). A videotape of these studies is available for viewing on the Harvard Skin Disease Research Center web page, under the Leukocyte Migration Core subheading (<http://dermatology.bwh.harvard.edu/leukocyte.html>).

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References

1. Stamper HB, Woodruff JJ. Lymphocyte homing into lymph nodes: *in vitro* demonstration of the selective affinity of recirculating lymphocytes for high endothelial venules. *J Exp Med.* 1976; 144:828–833. [PubMed: 956727]
2. Lawrence MB, Springer TA. Leukocytes roll on P-selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell.* 1991; 65:859–873. [PubMed: 1710173]
3. Fuhlbrigge RC, King SL, Dimitroff CJ, Sackstein R. Direct, real-time observation of E/and P/ selectin mediated rolling on cutaneous lymphocyte-associated antigen immobilized on Western blots. *J Immunol.* 2002; 168:5645–5651. [PubMed: 12023362]
4. Dimitroff CJ, Lee JY, Fuhlbrigge RC, Sackstein R. A distinct glycoform of CD44 is an L-selectin ligand on human hematopoietic cells. *Proc Natl Acad Sci USA.* 2000; 97:13841–13846. [PubMed: 11095749]
5. Dimitroff CJ, Lee JY, Raffi S, Fuhlbrigge RC, Sackstein R. CD44 is a major E-selectin ligand on human hematopoietic progenitor cells. *J Cell Biol.* 2001; 153:1277–1286. [PubMed: 11402070]
6. Fuhlbrigge RC, King SL, Sackstein R, Kupper TS. CD43 is a ligand for E-selectin on human skin homing T cells. *Blood.* 2006; 107:1421–1426. [PubMed: 16269612]
7. Hanley WD, Burdick MM, Konstantopoulos K, Sackstein R. CD44 on LS174T colon carcinoma cells possesses E-selectin ligand activity. *Cancer Res.* 2005; 65:5812–5817. [PubMed: 15994957]
8. Glasby, MA. Principles of haemodynamics. In: Glasby, MA., Huang, CL-H., editors. *Applied physiology for surgery and critical care.* Oxford & Associates; Boston, MA: 1995. p. 131
9. Sengers JV, Watson JTR. Improved international formulations for the viscosity and thermal conductivity of water substance. *J Phys Chem Ref Data.* 1986; 15:1291.
10. Borchers, H., editor. *Landolt-Bornstein Numerical Data and Functional Relationships in Physics, Chemistry, Astronomy, Geophysics, and Technology.* Vol. 4. Springer; Berlin: 1995. Material values and mechanical behaviour of non-metals.

Fig. 1. Diagram of blot rolling assay assembly. The components of the blot rolling assay are shown, including the inverted microscope, flow chamber, CCD camera, syringe pump, vacuum source, video monitor, and blot membrane (resting on a Petri dish located on the microscope stage, with the flow chamber positioned on top of it).

Fig. 2.

Blot rolling apparatus. Western blots stained for components of interest are equilibrated in binding media containing 10% glycerol and placed in a Petri dish. A parallel plate flow chamber mounted over the area of interest is held in place by low continuous vacuum pressure (Vac). The inlet (In) is connected to a buffer reservoir and the outlet (Out) to a high precision syringe pump. The apparatus is mounted on the stage of an inverted phase-contrast microscope equipped for videomicroscopy. Molecular mass(es) of interacting protein(s) resolved in the polyacrylamide gel (PAG) are estimated by alignment with colored mass standards in adjacent lanes (reproduced with permission from (3), Copyright 2002, The American Association of Immunologists, Inc.).

Fig. 3. Cutaneous lymphocyte antigen (CLA) immobilized on western blots supports both E-selectin and P-selectin mediated rolling. **(A)** Western blots of T cell lysates stained for CLA expression. 50 mg of cell lysate protein from CLA-positive (*left panel*) or CLA-negative (*right panel*) T cells were subjected to SDS-PAGE, electroblotted onto PVDF membranes and immunostained with HECA-452 mAb. The single major reactive glycoprotein identified at approximately 140 kDa in CLA-positive cells (lane 2) corresponds to the monomer form of CLA/PSGL-1. Similar blots stained with anti-PSGL-1 mAb show approximately equal quantities of 140 kDa PSGL-1 protein in each sample (data not shown). **(B and C)** Rolling cells per visual field (10× objective) at 1.75 dyne/cm² wall shear stress. The number of cells observed to bind and roll was observed in overlapping fields of view extending from approximately 250 to 40 kDa (identified alongside blot images in panel (A)). Both CHO-E and CHO-P cells are observed to tether and roll across the area of the western blot corresponding to CLA/PSGL-1 from CLA-positive T cells (panel (B)) while only CHO-P cells are noted to form significant rolling adhesions on PSGL-1 from CLA-negative T cells (panel (C)). Both CHO-E and CHO-P cells were noted to tether primarily over the area

corresponding to CLA/PSGL-1. Mock-transfected CHO cells did not form rolling tethers on any areas of the blots observed (data not shown). Results presented are the means of two independent experiments on a single substrate and are representative of observations on numerous blots of CLA-positive and CLA-negative T cell lysates (reproduced with permission from (3), Copyright 2002, The American Association of Immunologists, Inc.).

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