Regulatory Role of CD43 Leukosialin on Integrin-Mediated T-Cell Adhesion to Endothelial and Extracellular Matrix Ligands and Its Polar Redistribution to a Cellular Uropod

By Paloma Sánchez-Mateos, Miguel R. Campanero, Miguel A. del Pozo, and Francisco Sánchez-Madrid

CD43 is a cell surface-associated mucin that is abundantly expressed by most leukocytes, and that appears to function as a negative regulator of cell surface interactions, providing a repulsive barrier around cells. We have analyzed herein the ability of anti-CD43 monoclonal antibody (MoAb) to upregulate both β1 and β2 integrin-mediated cell adhesion and to promote redistribution of the CD43 molecule into a cellular uropod. Engagement of CD43 with specific antibodies enhanced the cell adhesion to both 80- and 38-kD fibronectin fragments as well as to the endothelial cell ligands vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, an effect that was mediated through the α5β1, α4β1, and αβ2 integrins, respectively. This effect on cell adhesion was achieved in Jurkat leukemic T cells by anti-CD43 MoAb alone; however, in T lymphoblasts, the activation of cell adhesion required the concomitant ligation of CD43 with suboptimal doses of anti-CD3 MoAb. Immunofluorescence analysis showed that the engagement of CD43 was accompanied by a differential redistribution of CD43 into a well-defined cytoplasmic projection or uropod, whereas the β1 or β2 integrins remained uniformly located on the contact area with substrata. This change in the localization of CD43 did not require costimulation and was induced directly by engagement of CD43 in T lymphoblasts. Other stimuli of cell adhesion in the form of cross-linked anti-CD3 MoAb or phorbol esters did not induce uropod formation or CD43 redistribution. In addition, we observed that prolonged coculture of resting peripheral blood T lymphocytes with endothelial cells, in the absence of anti-CD43 MoAb, induced uropod formation and redistribution of CD43 in T cells. Interestingly, the myosin-dissrupting drug butanedione monoxime inhibited the redistribution of CD43 induced by the specific MoAb, whereas the stimulation of cell adhesion induced by engagement of CD43 was preserved in the presence of this drug. These observations indicate that the signaling inducing integrin-mediated cell adhesion by CD43 takes place independently from the receptor redistribution. Altogether, these results indicate that CD43 has a regulatory role on both integrin-mediated T-cell adhesion and cellular morphology.

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T cells, but one anti-CD43 MoAb (L10) is capable of directly stimulating T-lymphocyte proliferation. Activation of T cells via CD43 involves the generation of diacylglycerol and inositol phosphates, with Ca2+ mobilization, and PKC activation. Because it is well known that factors that promote cell activation can enhance the avidity of an integrin for its ligand, it is possible that signals mediated through CD43 could trigger events that overcome the antiadhesion threshold provided by the same molecule.

We have herein explored the possibility that CD43 may regulate positively the adhesion ability of different integrin receptors. The results show that anti-CD43 MoAbs are capable of enhancing β1 and β2 integrin-mediated T-cell adhesion. Furthermore, the finding that CD43 is redistributed to a cell uropod suggests the existence of a mechanism by which the physical barrier that prevents interactions with other surface molecules might be reversibly modified.

### MATERIALS AND METHODS

**MoAbs.** Anti-CD43 HP2/21, MC7/1, TP1/15, TP1/36, TP1/51, TP1/57, and TP1/61 were obtained from fusions with splenocytes from mice immunized with the human leukemic JM cell line (HP2/21 and MC7/1 MoAbs) or with human T-lymphocytes activated for 24 hours with a combination of phorbol myristate acetate and anti-CD3 MoAb (TP series). Hybridoma supernatants were screened with a cell aggregation assay using the U937 cell line, and selected hybridomas producing aggregation-inducer antibodies were cloned twice by limited dilution. The anti-CD43 L10, and 84-3C1, anti-ICAM-1 RR1/1,29 anti-ICAM-3 TP1/24, anti-LFA-1 (CD11a) TS1/11, anti-LFA-1/3 (CD18) Lia 3/2,30 anti-CD29 TS2/16,30 anti-CD49d HP2/1,32 anti-CD49e SAM-1,32 and anti-CD3 SPV-T3b34 MoAbs have been described. Anti-CD45 conjugated to fluorescein isothiocyanate (FITC) was purchased from Becton Dickinson (Mountain View, CA). For cell adhesion experiments, MoAbs were purified from ascites fluid with protein A coupled to Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden). P3X63 myeloma culture supernatant was used as negative control.

**Protein substrate and reagents.** Recombinant chimeric vascular cell adhesion molecule-1-Fc (VCAM-1-Fc) and recombinant soluble E-selectin were kindly provided by Dr R. Lob (Biogen, Cambridge, MA). Recombinant chimeric ICAM-1-Fc, consisting of the total extracellular domains fused to IgG1 Fc fragment was obtained as previously described. Briefly, COS-7 cells were transiently transfected with the plasmid CAM-I-Fc (ICAM-1 cDNA cloned in pCD8IgGl). After 4 days, culture supernatants were precipitated with ammonium sulphate, and, thereafter, ICAM-1-Fc was isolated by using protein A coupled to Sepharose (Pharmacia). The tryptic 38-kD fibronectin (FN40) and 80-kD fibronectin (FN80) fragments were kindly provided by Dr A. García-Pardo (Centro de Investigaciones Biológicas, Madrid, Spain). Fibrinogen was purchased from Sigma (St Louis, MO). Cytochalasin D, colchicine, and butanedione monoxime were also purchased from Sigma.

**Cells and cell lines.** Resting peripheral blood lymphocytes (PBLs) were isolated from fresh human blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia), followed by two steps of adherence incubation on plastic flasks (Costar, Cambridge, MA) at 37°C for 1 hour. Human T lymphoblasts were prepared from PB mononuclear cells (PBMCs) by treatment with phytohemagglutinin (0.5%; Pharmacia) for 48 hours. Cells were washed and cultured in RPMI 1640 (Flow Laboratories, Irvine, Scotland) containing 10% fetal calf serum (FCS; complete medium; Flow Laboratories) and 20 U/mL interleukin-2 (IL-2) kindly provided by Eurocetus (Madrid, Spain). T lymphoblasts cultured by 7 to 12 days were typically used in all experiments. T lymphoblasts and T-cell clones have been extensively used to study both LFA-1-mediated cell adhesion and T-cell activation. The Jurkat T-cell line was maintained in RPMI 1640 plus 10% FCS.

Human umbilical vein ECs (HUVECs) were obtained as described. Briefly, the umbilical vein was cannulated, flushed, and incubated with 0.1% collagenase P (Boehringer Mannheim GmbH, Mannheim, Germany) for 20 minutes at 37°C. Cells were seeded into flasks and cultured in M199 medium (Flow Laboratories) supplemented with 20% FCS, 50 µg/mL EC growth supplement, and 100 µg/mL heparin (Sigma). EC cultures were split 1:3, and, before using, the cells were detached with a solution of 0.05% Trypsin and 0.02% EDTA (Flow Laboratories). Cells within two passages were used in all experiments.

**Immunoprecipitation and electrophoresis.** PBMCs were radio-labeled and lysed in lysis buffer (phosphate-buffered saline, pH 7.4, containing 1% Triton X-100, 1% hemoglobin, and 1 mmol/L phenylmethylsulfonyl fluoride). Equal amounts of 125I-labeled cell lysates were mixed with 100 µL of MoAb-containing culture supernatant, and immune complexes were isolated adding 100 µL of 187.1 antimouse M-chain MoAb followed by 30 µL protein A coupled to Sepharose. Immunoprecipitates were processed as described and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography.

**Cell adhesion assays.** Adhesion assays were essentially performed as previously described. Briefly, microtiter EIA Linbro plate (Costar) wells were incubated with 50 µL/well of 20 mmol/L Tris-HCl (pH 8.0) containing either 5 µg/mL or 20 µg/mL of FN40 or FN80 fragments, respectively, or 5 µg/mL of recombinant chimeric VCAM-1-Fc or ICAM-1-Fc for 2 hours at 37°C. Then, the plates were saturated with 1% human serum albumin and washed, and either 2 or 3 x 105 (T lymphoblasts) or 1 x 105 (Jurkat cells)/well in 100 µL were added and centrifuged for 5 minutes at 10g before an incubation at 37°C for 10 to 20 minutes. To quantify cell attachment, the plates were washed with RPMI containing 0.5% human serum albumin, fixed with a mixture of acetone/methanol 1:1, and dyed with violet crystal 0.5%. The absorbance at 540 nm was measured in an enzyme-linked immunosorbent assay reader (LP400; Kallestad, Chaska, MN), and the number of cells was calculated. The optical density was found to be a linear function of the number of cells by a calibration curve (optical density versus number of cells). Total cellular input was calculated by spinwashing wells with the original number of cells, staining, and measuring optical density.

**Immunofluorescence.** Immunofluorescence experiments were performed as previously described. Briefly, 2 x 105 T lymphoblasts were incubated in flat-bottomed, 24-well plates (Costar) in a final volume of 500 µL of complete medium or coverslips coated either with 20 µg/mL of FN40 or FN80 fragments or with 10 µg/mL VCAM-1-Fc or ICAM-1-Fc. For some experiments, HUVECs were grown on gelatin-coated coverslips and stimulated with 10 ng/mL tumor necrosis factor-α (TNF-α) for 16 hours at 37°C; then, PBLs were incubated on these coverslips. Cells were incubated on coverslips and MoAbs were added at a final concentration of 1 µg/mL, and cells were allowed to settle in a cell incubator at 37°C and 5% CO2 atmosphere. After 30 minutes, cells were fixed with 3.7% formaldehyde in phosphate-buffered saline for 10 minutes at room temperature and rinsed in TBS (50 mmol/L Tris-HCl [pH 7.6], 150 mmol/L NaCl, 0.1% Na3), To visualize the stimulatory MoAbs bound to the cells, the coverslips were stained with a 1:50 dilution of an FITC-labeled rabbit F(ab')2, antimouse IgG (Pierce, Rockford, IL). For double-label studies, after staining the stimulatory MoAb with FITC-secondary antibody, cells were saturated with 10% non-specific mouse serum in TBS. Then, cells were incubated with biotinylated MoAbs to other proteins, followed by washing and labeling with tetramethyl rhodamine isothiocyanate (TRITC)-avidin D (Vec-

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tor, Burlingame, CA) for 30 minutes. The cells were then washed with TBS and incubated with a biotinylated anti-avidin (Vector) for 30 minutes. Finally, a fourth incubation with TRITC-avidin D for 30 minutes was performed. The cells were observed in a Nikon Labophot-2 photomicroscope (Nikon, Tokyo, Japan) with a 60X oil immersion objective and were photographed on either ektachrome 400 (color) or TMAX 400 (black and white) film (Eastman Kodak Co, Rochester, NY). The latter was processed to 800 to 1600 ASA with TMAX developer (Eastman Kodak). Where indicated, red and green fluorescence was photographed on the same frame, and, in some cases, we were compelled to adjust the focus to show both colors in focus.

RESULTS

Characterization of the specificity of novel anti-CD43 MoAbs. Several MoAbs (HP2/21, MC7/1, TP1/3, TP1/36, TP1/51, TP1/57, and TP1/61) were selected initially for their ability to induce a rapid and strong aggregation of both U-937 myelomonocytic cells and normal leukocytes. These MoAbs immunoprecipitated a polypeptide of 115 kD from ¹²⁵I-labeled PBMNC lysates (Fig IA, lanes 2 through 8). The immunoprecipitation and the cellular distribution patterns of the antigen recognized by these novel MoAbs were identical to the human CD43 antigen (Fig IA, lanes 2 through 8, and data not shown). The novel MoAbs TP1/36 and MC7/1, as well as the previously described anti-CD43 L10 MoAb (Fig IB, lanes 2, 3, and 4, respectively). Sequential immunoprecipitation experiments of these new MoAbs with the anti-CD43 L10 MoAb further showed that each one recognizes the CD43 antigen (data not shown).

Effect of anti-CD43 MoAbs on cell adhesion to purified ligands. The triggering of homotypic leukocyte aggregation, as well as the induction of stimulatory cytoplasmic signals, by anti-CD43 MoAbs have previously been described. We have now explored the potential effect of CD43 as regulator of cell-cell and cell-ligand interactions mediated by integrins. To this end, cell-binding assays to different anti-CD43 MoAbs. As shown in Fig 2, the anti-CD43 TP1/36 MoAb was able to enhance T-lymphoblast attachment to the FN80 fragment that contains the Arg-Gly-Asp peptide ($P < 0.05$). This effect was significantly increased when the anti-CD43 MoAb was combined with a very low dose of anti-CD3 MoAb, which by itself was unable

![Fig 1. Characterization of seven novel anti-CD43 MoAbs. ¹²⁵I-labeled cell lysates from PBMNCs were immunoprecipitated with the following MoAbs: control P3X63 (A and B, lane 1) and anti-CD43 HP2/21, MC7/1, TP1/3, TP1/36, TP1/51, TP1/57, and TP1/61 (A, lanes 2 through 8); and TP1/36, MC7/1, and L10 (B, lanes 2 through 4). Immune complexes were isolated, and reduced samples were subjected to sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis and autoradiography.

![Fig 2. CD43-mediated induction of cell adhesion to FN80. T lymphoblasts were pretreated for 30 minutes at 4°C with a combination of different doses of the anti-CD3 T3b MoAb with medium alone (○), anti-CD43 TP1/36 (10 µg/mL; ■), or anti-ICAM-3 TP1/24 (10 µg/mL; ▲) MoAbs. Next, 10 µg/mL sheep antimouse IgG was added as a cross-linker for 10 minutes at 4°C. Adhesion to FN80-coated microtiter wells was quantified after 10 minutes of incubation at 37°C. Arithmetic mean ± 1 SD of triplicate wells is shown. Data are representative of five experiments. Adhesion to albumin-coated wells was always lower than 5%.]
Fig 3. CD43-mediated induction of cell adhesion to either FN80, FN40, VCAM-1, or to ICAM-1 by different anti-CD43 MoAbs. T lymphoblasts were pretreated for 30 minutes at 4°C with the following stimuli: RPMI (Medium), 10 μg/mL of anti-ICAM-3 TP1/24, anti-CD43 TP1/36, 84-3C1, and L10 MoAbs; in the absence (●) and in the presence (■) of 0.05 μg/mL of anti-CD3 T3b MoAb. Next, 10 μg/mL sheep antimouse IgG was added as a cross-linker for 10 minutes at 4°C. Adhesion to either FN80-, FN40-, VCAM-1-, or ICAM-1-coated microtiter wells, as indicated, was quantified after 10 minutes of incubation at 37°C. Arithmetic mean ±1 SD of triplicate wells is shown. Data are representative of six experiments. Adhesion to albumin-coated wells was always lower than 5%.

The CD43-induced cell binding to FN80, FN40, and VCAM-1 was specifically inhibited by MoAbs to very late-activation antigen-5 (VLA-5) and to VLA-4, respectively (Fig 4A). These data indicate that CD43 is able to regulate the interaction of VLA-5 and VLA-4 integrins with their respective ligands. Furthermore, the CD43-stimulated binding of T lymphoblasts to ICAM-1 was also completely inhibited by the anti-LFA-1α TS1/11, the anti-LFA-1β Lia 3/2, and the anti-ICAM-1 RR1/1 MoAbs (Fig 4B), thus indicating that CD43-induced lymphoblast adhesion to ICAM-1 is specifically mediated by the LFA-1 integrin.

The conditions required for activation of adhesion via CD43 were further investigated using the Jurkat T-cell line. Several anti-CD43 MoAbs were able to directly enhance Jurkat cell binding to the FN80 fragment in the absence of anti-CD3 MoAb (Fig 5). This finding confirms that, in certain cells, the engagement of CD43 alone, in absence of co-occupancy of other membrane receptors, is able to enhance cell adhesion.

Cell surface redistribution of CD43 accompanies T-cell...
It was of interest to study whether the binding of anti-CD43 MoAb would have any effect on cell morphology or in cell surface molecule reorganization. To this end, the localization of CD43 and β1 integrins in cells adhering to both FN80 and VCAM-1 was simultaneously analyzed by two-color immunofluorescence microscopy (Fig 6). Most T lymphoblasts in suspension showed a uniform distribution of CD43, and, in only a small fraction of the cells (usually less than 10%), CD43 was polarized to one region of the cell membrane (data not shown). Similarly, the distribution of CD43 on cells adhering to different β1-integrin ligands was uniform over the cell surface and appeared to colocalize with β1 integrins (Fig 6G through I). Very interestingly, during CD43-induced cell attachment to the FN80 fragment and to VCAM-1, dramatic changes in both the cell surface distribution of CD43 and cell morphology were observed (Fig 6A and D, respectively). Anti-CD43 MoAb induced redistribution of the antigen toward a well-defined cytoplasmic projection or uropod. The uropod in which CD43 was concentrated was observed in an upper focal plane, distant from the portion of the cell membrane that was in contact with the substrate, in which the β1 integrins were located (Fig 6B and E, red fluorescence).

The results shown above strongly suggested that the LFA-1/ICAM-1 interaction was mediating the CD43-induced cell adhesion to ICAM-1. To further explore the role of CD43 in cell adhesion to ICAM-1, we studied the localization of CD43 by immunofluorescence analysis of T lymphoblasts allowed to interact with ICAM-1-coated coverslips. Unstimulated T lymphoblasts interacting with ICAM-1 showed a uniform distribution of CD43 all over the cell surface (Fig 7A). Interestingly, the treatment with the anti-CD43 TP1/36 MoAb also induced both, a change in cell morphology with the appearance of a cell uropod and the clustering of the molecule in this structure (Fig 7B), whereas the cellular distribution of other membrane glycoproteins such as CD45 remained unchanged (Fig 7C). Thus, most of the CD43 molecules were located into the cell uropod that emerged out of the contact area with the substrate. In contrast, LFA-1 was...
uniformly distributed over the cell contact area with the ICAM-1-coated surface (data not shown), in a manner similar to the distribution of β1 integrins during the adhesion to FN-coated or VCAM-1-coated surfaces.

The recruitment of CD43 to the uropod in T lymphoblasts was directly induced by the anti-CD43 MoAb, in the absence of additional cross-linkings (Fig 7B). The activation of adhesion via CD43 in T lymphoblasts that required the cross-linking of CD43 and CD3/Tcr also resulted in polarization of CD43 to the cell uropod (Fig 7D). In contrast, other stimuli of cell adhesion such as cross-linked anti-CD3 MoAb, which induced the extension of filopodia on the substrate, or phorbol esters, which promoted a symmetrical spreading of the cells, did not induce uropod formation or CD43 redistribution (Fig 7E and F, respectively).

Redistribution of CD43 during the adhesion of lymphocytes to HUVECs. In addition to the use of purified integrin ligands to test the induction of cell polarization via CD43, we also monitored the CD43 distribution on lymphocyte-EC contact. When resting PBLs were incubated during 30 minutes with unstimulated or cytokine-activated HUVECs in the presence of anti-CD43 MoAb, most of the PBLs showed a CD43 redistribution to distinctive cell uropods (data not shown). Lymphocyte-EC contact had no immediate effect on polarization of resting PBLs in the absence of anti-CD43 MoAb, when tested in a 30-minute incubation (<10% polarized cells; data not shown). However, with longer time period of lymphocyte-EC coculture, the proportion of cells bearing uropodia in the absence of CD43 stimulation increased. Thus, after 24 hours of coculture, more than 50% of lymphocytes showed a polarized morphology with a clear uropod formation, where CD43 was partially redistributed (Fig 8A and B). Both resting and TNF-α-activated ECs promoted the development of uropods in lymphocytes after overnight coculture (Fig 8A and B, respectively).

The modulation of integrin-mediated T-cell adhesion by CD43 does not require redistribution of CD43. We have previously described the involvement of the myosin motor in the redistribution of other adhesion molecules, such as ICAM-3, to the cell uropod.43 Therefore, we have investigated the involvement of cytoskeletal components in the regulation of cell morphology and molecule redistribution induced by CD43. To this end, we have used several drugs that specifically inhibit different cytoskeletal components. The myosin-disrupting drug butanedione monoxime prevented the redistribution of CD43 induced by the specific MoAb (Fig 9B), suggesting that the forces driving cell polarization in response to CD43 stimulation may be dependent on the myosin motor. Interestingly, the stimulation of integrin-mediated cell adhesion induced by CD43 was preserved in the presence of this drug (Fig 9D), indicating that the signaling inducing integrin-mediated cell adhesion by CD43 is independent from the polarization response. Disruption of microtubules with colchicine did not affect the polarization response or the increment in cell adhesion induced by CD43 stimulation (Fig 9C and D, respectively). The disruption of actin microfilaments with cytochalasin D completely inhibited cell adhesion to the substratum (Fig 9D).

**DISCUSSION**

Although recent works on CD43 indicate that this molecule would function as a negative barrier that limits non-specific cell-cell interactions,14,15 we have found herein that CD43 may also function as a positive signaling molecule for some specific adhesion receptors such as β1 and β2 integrins. In addition, immunofluorescence analysis showed that CD43 stimulation induces the polarization of the molecule towards an apical cell uropod, whereas integrins are uniformly distributed on the cell-substrate contact area.

The data presented here suggest that the activation of cell adhesion induced through CD43 is a selective phenomenon that affects only some adhesion receptors. The increment in cell adhesion to β1 and β2 integrin ligands but not to E-selectin does not support the induction of an unspecific mechanism, such as the disruption of a physical barrier, and suggests the selective activation of certain receptors through CD43. Initial studies of CD43 indicated the ability of anti-CD43 MoAb to induce homotypic cell adhesion of different hematopoietic cells.19,21 Recently, CD34, another member of the mucin-like family, has also been implicated in cell aggregation.44 It is possible that the natural ligand(s), mimicked by anti-CD43 MoAb, may transduce activation signals to specific adhesion receptors. Accordingly, it has been described that the signal transduction through CD43 involves the generation of diacylglycerol and inositol phosphates, Ca²⁺ mobilization, and PKC activation,22,24 signals that usu-

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**Fig 5.** CD43-mediated induction of Jurkat cell adhesion to FN80 in the absence of CD3-TcR stimulation. Jurkat cells were pretreated for 30 minutes at 4°C with the following stimuli: RPMI (Medium), 10 μg/mL of anti-CD43 TP1/36, 84-3C1, and L10, and anti-ICAM-3 TP1/24 MoAbs. Adhesion to FN80-coated microtiter wells was quantified after 7 minutes of incubation at 37°C. Arithmetic mean ± SD of triplicate wells are shown. Data are representative of five experiments. Adhesion to albumin-coated wells was always lower than 5%.
ally results in integrin activation. One important consequence of this signaling could be to exceed a critical activation threshold for cell adhesion provided by large and prevalent anionic surface molecules such as CD43. Thus, CD43 may function both as a physical barrier that limits cell interactions and as a cell receptor that stimulates cell adhesiveness. Our data provide the first indication that the interaction of the extracellular domain of CD43 with a naturally occurring ligand(s) might regulate the cell adhesion mediated by both β1 and β2 integrins.

Interestingly, the polarization response and the clustering of CD43 to the cell uropod are specifically induced through CD43 stimulation. Other stimuli that enhance integrin-mediated cell attachment differ in their ability to promote morphological changes, but do not involve the clustering of CD43 into a cellular uropod. Thus, the clustering of CD43 out of
Fig 7. CD43 induces specifically the redistribution of CD43 to the cell uropod, but not other stimuli of integrin-mediated adhesion. T lymphoblasts were allowed to bind to coverslips coated with 10 μg/mL sICAM-1 for 30 minutes at 37°C either (A) in the absence of any stimuli or (B and C) in the presence of 10 μg/mL of the anti-CD43 TP1/36 MoAb; (D) 10 μg/mL of the anti-CD43 TP1/36, plus 0.05 μg/mL of anti-CD3 T3b MoAb, plus 10 μg/mL sheep antimouse IgG, as a cross-linker (adhesion conditions); (E) 10 μg/mL of the anti-CD3 T3b plus 10 μg/mL sheep antimouse IgG, as cross-linker; or (F) 20 ng/mL phorbol myristate acetate. Then, fixed cells were stained as described in the Materials and Methods section, either for CD43 by using biotinylated anti-CD43 MoAb (A, B, D, E, F) or for CD45 by using an FITC-labeled anti-CD45 MoAb (C). (Bar, 10 μm.)

Fig 8. Morphological changes and CD43 redistribution during interaction of PBLs with ECs. PBLs were cultured, for 24 hours, at 37°C, over coverslips coated with HUVECs that were either unstimulated (A) or TNF-α-stimulated (B; 10 ng/mL TNFα for 16 hours at 37°C). Then, fixed cells were stained for CD43 as described in the Materials and Methods section. Same fields were photographed under epifluorescent (left panels) and bright field (right panels) conditions. (Bar, 20 μm.)
the contact area with the substratum is not a prerequisite of any integrin-mediated cell adhesion induced through different stimuli. Moreover, it is possible to dissociate the different effect of CD43 stimulation in both integrin-mediated cell adhesion and CD43 polarization. Thus, in the presence of the myosin-disrupting drug butanedione monoxime, the raising of the cell uropod and the molecule redistribution are completely inhibited, but the CD43-induced enhancement of cell adhesion is not. The mechanism by which CD43 stimulates cell adhesion may be related to an activation of integrin avidity and may not simply be because of a reduced repulsion in the area of the membrane free of CD43.

The clustering of CD43 into the cell uropod could be a part of a regulatory mechanism that controls the surface expression of the molecule. The first indication that CD43 is downregulated in vivo came from the analysis of its expression in neutrophils from patients undergoing hemodialysis therapy with Cuprophane membranes. In addition, the downregulation of CD43 expression on activated neutrophils from inflammatory synovial fluids was recently reported. It has also been found that CD43 is proteolytically cleaved from the surface of neutrophils in response to different in vitro stimuli. Further work in neutrophils showed that albumin inhibits the shedding of CD43 and that there are two independent proteolytic pathways for CD43 downregulation. In addition, a soluble galactoglycoprotein identical to the extracellular domain of CD43 is detected in high concentrations in human serum, indicating that the cleavage of membrane CD43 may take place in vivo, thus producing galactoglycoprotein. The cleavage of CD43 from the surface of lymphocytes has also been described; however, longer periods of time were required than in neutrophils. Therefore, it is conceivable that alternative mechanisms may exist by which CD43 activity might be reversibly modified in lymphocytes (including ligand binding, phosphorylation, or other covalent modifications, molecular interaction with other surface molecules or cytoskeletal components) that lead to a redistribution of CD43 in the cell membrane. The redistribution of a membrane receptor would allow the rapid modulation of the molecule, a phenomenon that might be faster and most readily reversible than the enzymatic cleav-
age of the receptor. The redistribution of CD43 within the uropod could be part of a multistep regulatory system of CD43 downregulation in lymphocytes. First, physiological stimuli, such as the interaction with natural ligands or chemotactic cytokines, should elicit a rapid surface redistribution of CD43 towards the uropod, and, second, prolonged stimulation should lead to proteolytic cleavage of CD43.

An additional possibility is that the induced surface redistribution of CD43 may be part of a regulatory mechanism controlling the activity of certain leukocyte surface molecules. Thus, it has long been reported that activation of lymphocytes is accompanied by changes in the morphology of the cell; ie, spherical resting T cells become polarized, bearing a well-defined cytoplasmic projection or uropod. Interestingly enough, ICAM-1, a ligand of LFA-1 that also appears to interact with CD43, is located within the uropod in certain lymphoblastoid cell lines. In fact, recent studies have shown that ICAM-3, the most abundantly expressed LFA-1 counter-receptor on leukocytes, is also driven into the uropod on specific MoAb ligation. ICAM-3 shares several features with CD43: (1) it is also a heavily glycosylated membrane protein restricted to leukocytes; (2) it is downregulated from the surface of activated neutrophils by a proteolytic shedding mechanism; and (3) antibody ligation of ICAM-3 also results in an increase of the avidity of certain integrins for their ligands. A recent study has shown that lymphocyte interactions with ECs induce uropod formation where CD2, L-selectin, and CD44 are concentrated. Thus, it could be postulated that several cell surface molecules may be driven into the uropod by specific stimuli or after interaction with natural ligands expressed by ECs. In this regard, our finding on uropod formation after prolonged PBL-EC interaction and the CD43-relocation to it supports this issue. Moreover, the nature of the cytoplasmic connections that drive these molecules into the uropod might represent a general mechanism regulating surface distribution of these molecules. In this respect, the molecular interaction of CD43 through its cytoplasmic domain with actin-based cytoskeleton has been recently described. Accordingly, the preferential accumulation of myosin into the uropod region has also been described during the ICAM-3-regulated formation of this cellular structure. Furthermore, our data also suggest that the myosin motor is involved in the redistribution of CD43 to the cell uropod. Therefore, it will be of interest to investigate in detail the cytoplasmic components involved in the cellular regional polarization and the precise physiological effect of the redistribution of highly charged cell surface glycoproteins that are similar to CD43.

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P Sanchez-Mateos, MR Campanero, MA del Pozo and F Sanchez-Madrid