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CD73 Engagement Promotes Lymphocyte Binding to Endothelial Cells Via a Lymphocyte Function-Associated Antigen-1-Dependent Mechanism

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CD73 is a GPI-anchored lymphocyte adhesion molecule possessing an ecto-5'-nucleotidase enzyme activity. In this work, we show that engagement of lymphocyte CD73 increases lymphocyte binding to cultured endothelial cells (EC) in an LFA-1-dependent fashion. Engagement of CD73 by an anti-CD73 mAb 4G4 increases the adhesion of lymphocytes to cultured EC by about 80% compared with that of lymphocytes treated with a negative control Ab, and the increased adhesion can be blocked by an anti-CD18 mAb. The CD73-regulated increase in lymphocyte adhesion is not due to a conformational change leading to high-affinity LFA-1 receptors as assayed using mAb 24 against an activation-induced epitope of the molecule. Instead, CD73 engagement induces clustering of LFA-1 that is inhibitable by calpeptin, indicating involvement of Ca\(^{2+}\)-dependent activation of a calpain-like enzyme in this process. In conclusion, the results shown here demonstrate that CD73 regulates the avidity of LFA-1 by clustering. This indicates a previously undescribed role for CD73 in controlling the poorly characterized activation step in the multistep cascade of lymphocyte extravasation. Moreover, these results suggest that in physiological conditions the activation step may result in clustering of LFA-1 rather than in an affinity change of the molecule. The Journal of Immunology, 2000, 165: 5411–5417.
CD73 ENGAGEMENT INDUCES LYMPHOCYTE ADHESION TO EC

Identical CD73 cDNA sequences in different tissues (25). Incubation of lymphocytes with the anti-CD73 mAb 4G4 at a physiologival temperature of 37°C causes shedding of CD73, but EC CD73 is not shed due to anti-CD73 mAb treatment (25). Moreover, engagement of lymphocyte CD73 results in tyrosine phosphorylation and dephosphorylation of intracellular protein substrates, whereas CD73 on EC remains resistant to mAb engagement in this respect (25). These differences in regulation and function suggest differences in the physiological role of the molecule in various sites in the body. Although these regulatory aspects we previously reported the involvement of CD73 in lymphocyte-EC interactions (22), the mechanisms of its action have remained completely unknown. Therefore, this work was designed to elucidate the mechanisms by which CD73-controlled lymphocyte-EC interactions are conducted.

Materials and Methods

Cells, cell lines, Abs, and other reagents

The EAhy-926 cell line (26) (HEC) was cultured in RPMI 1640 medium supplemented with 10% FCS, 4 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. HUVEC were isolated and cultured as described previously (22). Human PBL were isolated using Ficoll-Hypaque (His-topaque-1077, Pharmacia, Uppsala, Sweden). mAb 4G4 (mouse IgG1) recognizes CD73 (21). Hybridoma cell lines producing mAbs against CD18 (HB116, mouse IgG1), and MHC class I (HB95, mouse IgG1) were obtained from American Type Culture Collection (Manassas, VA). Anti-CD73 mAb 1E9 was a gift from Linda Thompson (Oklahoma Medical Research Foundation, Oklahoma City, OK), and mAb 24 recognizing an epitope present on LFA-1 at an active state was a gift from Nancy Hogg (Imperial Cancer Research Fund, London, U.K.). The production of anti-ICAM-1 mAb 5C3 (mouse IgG1) was described previously (20). Irrelevant isotype-matched mAbs against chicken T cells (3G6, mouse IgG1) and human vascular adhesion protein-1 (2D10, mouse IgG1), biotinylated or conjugated to FITC, were used as negative control Abs. FITC-conjugated anti-CD11a and anti-CD18 were purchased from Immuno-technet (Marseille, France), FITC-conjugated anti-CD8 and streptavi- din-PE were obtained from Becton Dickinson (San Jose, CA). FITC-con- jugated sheep anti-mouse Ig, CBZ-Leu-Val-Gly diazomethyl ketone (CBZ), and PMA were purchased from Sigma (St. Louis, MO). Fluoro- mount was obtained from Southern Biotechnology Associates (Birmingham, AL) and ionomycin (Streptomyces conglutobates) and calpeptin were purchased from Calbiochem-Novabiochem (La Jolla, CA).

Adhesion assays

In assay A, monolayers of HEC cells or HUVEC were cultured on 96-well tissue culture plates until confluence was reached. Before the assay, the monolayers were incubated with PBS/1% BSA for 20 min at 4°C. Freshly isolated PBL were fluorescence labeled as described previously (22), then 10^5 PBL were resuspended in 100 μl of ice-cold mAb 4G4, 3G6, HB116, or HB203 as tissue culture supernatant, supplemented with 5% human AB serum, and the additional incubations and washing procedures were performed as described for assay A. In assay C, adhesion assays were performed as described for assay A, but 15 min before the end of bis-carboxyethyl carboxyfluorescein labeling, 10 ng/ml PMA was added, and additional incubation steps were performed in the presence of PMA.

In assay D, adhesion assays were performed as described for assay A, but calpeptin and CBZ were added to the tubes in a final concentration of 100 μM 15 min before mAb incubations. Calpeptin and CBZ were present until cells were stained and fixed.

Results are expressed as relative adhesion ratios ± SEM. A value of 1.0 is given to the binding efficiency in the presence of a negative control Ab, and binding in the presence of other experimental conditions is compared with that.

Detection of the activation-induced epitope 24

To determine whether the increased adhesion between lymphocytes and EC is due to increased activation of LFA-1 after mAb 4G4 treatment, immunofluorescence analyses using mAb 24, which recognizes LFA-1 in a high-affinity state, were performed. Lymphocytes (1.2 × 10^6) were suspended with regular RPMI 1640 (containing Ca^{2+} and Mg^{2+}) or HBSS without Ca^{2+} and Mg^{2+} containing 20 μg/ml mAb 4G4, 20 μg/ml of a class-matched negative control Ab (3G6), or 1 mM Mn^{2+}, which was used as a positive control because it is known to cause activation of LFA-1 (27). Cells were then warmed and incubated at 37°C in a humidified incubator for 30 min to cause 4G4-induced shedding of CD73. Activation was assayed by incubating the cells for 20 min with biotinylated mAb 24 at 37°C and with a biotinylated negative control Ab (2D10) followed by streptavidin-PE.

A similar number of cells was left untreated and stained immediately after isolation on ice with Abs against CD73 and epitope 24 or with a negative control Ab to determine the expression levels before the treatments. After staining, the cells were put on ice and washed twice with ice-cold PBS containing 2% FCS and 0.1% NaN3, and fixed with 1% form- aldehyde. The surface expression before and after treatment was analyzed using a FACScan cytometer (Becton Dickinson, Mountain View, CA).

Confocal microscopy

To observe possible clustering of cell surface LFA-1 molecules after engagement of CD73, immunofluorescence assays were performed. Freshly isolated lymphocytes were used. To achieve CD73 engagement on as many lymphocytes as possible, lymphocytes from healthy volunteers known to have high expression of CD73 (range, 15–20%) were used.

In brief, 8 × 10^3 cells in 400 μl of RPMI 1640 were incubated in plastic tubes. A final concentration of 100 μg/ml calpeptin, a specific membrane-soluble inhibitor of the cytosolic protease calpain, was added to the tubes. Equal number of tubes without calpeptin served as controls. A protein G-purified mAb 4G4 against CD73 or a negative control Ab 3G6 (final concentration, 20 μg/ml) and AB serum (final concentration, 5%) were added to the cells were incubated for 30 min on ice. Cells were warmed to 37°C and then incubated for 30 min at 37°C to cause mAb 4G4-triggered shedding of CD73 from the cell surface. Cells were then put on ice and washed twice with ice-cold PBS containing 2% FCS and 0.1% NaN3.

Immunofluorescence staining was performed using FITC-conjugated mAbs against CD11a, CD18, and D8. An irrelevant FITC-conjugated mAb was used as a negative control. Cells were incubated for 20 min at 4°C with these FITC-conjugated mAbs containing 5% AB serum. Cells were washed once with PBS containing 2% FCS and 0.1% NaN3, and once with PBS containing 0.1% NaCN, and fixed with formaldehyde. All washing media contained 0.1% NaN3, to prevent Ab-induced clustering of LFA-1. A Ca^{2+} mobilizer, ionomycin (1 μM), that has been reported to induce clustering was used as a positive control (28). After treatment and staining the cells were spun onto glass slides at 1000 rpm for 5 min. Cells were left to dry for 10 min and thereafter were mounted with Fluoromount. Cells were analyzed using a confocal microscope (Leica TSC 4D confocal system connected to a Leica RXA microscope; Leica Microsystems, Heidelberg, Germany). Instrument settings were kept constant in all samples within individual experiments.

Results

Engagement of CD73 enhances lymphocyte binding to endothelium

Freshly isolated PBL and either in vitro cultured HEC cells or HUVEC were used in the present study to investigate the contribution of the CD73 molecule in regulating lymphocyte adhesion to EC. Anti-CD73 mAbs have been shown to be involved in regulating lymphocyte adhesion (21, 22, 29), but the exact mechanism of CD73 function has remained unclear. Under the experimental conditions used here, engagement of lymphocytes with anti-CD73
mAb 4G4 caused an increase in lymphocyte binding to EC (Fig. 1A). In the presence of a negative control mAb HB116 (anti-HLA-ABC; mouse IgG1) about 25% of freshly isolated PBL adhered to cultured EC. The presence of anti-CD18 mAb HB203 caused a 23% inhibition of lymphocyte binding compared with mAb HB116 (Fig. 1A; n = 11; p = 0.0057). Adding anti-CD73 mAb 4G4 to the assay caused an 82% increase in lymphocyte binding under the same conditions, with 38% of all input lymphocytes bound to EC (Fig. 1A; n = 17; p = 0.0001, 4G4 vs HB116). These experiments were repeated using HUVEC instead of HEC cells, and the results were similar, i.e., in the presence of anti-CD73 mAb 4G4, PBL adhered more avidly to HUVEC than in the presence of an anti-HLA-ABC mAb (Fig. 1B). Lymphocyte binding to HUVEC was also reduced by the anti-CD18 mAb (Fig. 1B). In this assay, it is essential to allow the lymphocytes to settle onto the EC monolayers in the cold with the Abs maximally bound to the molecules, because if the lymphocytes are exposed to a warm temperature in the presence of the 4G4 Ab, shedding of CD73 occurs prematurely, and an opposite effect on lymphocyte adhesion is observed, as shown previously (22, 29). In some experiments PMA, a potent enhancer of LFA-1-mediated binding, was used as a positive control (Fig. 1C). Interestingly, the binding level following PMA treatment (40%) was similar to the level seen after stimulation with CD73 engagement alone regardless of whether CD73 engagement was performed simultaneously with PMA stimulation.

To test the effect of another anti-CD73 mAb, 1E9, on lymphocyte adhesion, similar experiments were repeated using this Ab. Surprisingly, no increase in lymphocyte binding to EC was observed following 1E9 treatment, whereas the increase in binding due to 4G4 mAb treatment was >2-fold compared with that using negative control mAb 3G6 (Fig. 1D; n = 4; p = 0.0001). As 1E9 and 4G4 are known to recognize distinct epitopes on CD73, and mAb 4G4 causes shedding of CD73, but 1E9 fails to do this (25), this result suggests that shedding of CD73 from the lymphocyte surface might be essential in regulating lymphocyte adhesion, and it takes place subsequent to ligand binding to the same region recognized by 4G4 mAb.

**FIGURE 1.** Increased lymphocyte adhesion to EC after CD73 engagement with 4G4 mAb. Freshly isolated PBL were allowed to adhere to EC monolayers in the presence of different Abs as described in Materials and Methods. Results in A, B, and D are expressed as the relative adhesion ratio, where the relative adhesion ratio in the presence of HB116 = 1. A, Lymphocytes were allowed to adhere to HECs in the presence of anti-HLA-ABC mAb HB116, anti-CD18 mAb HB203, and anti-CD73 mAb 4G4. B, Lymphocytes were allowed to adhere to HUVECs in the presence of mAb HB116, mAb HB203, and mAb 4G4. C, The binding assay was performed as described in A, with PMA as a positive control for LFA activation. The data are shown as the percentage of bound cells of all input cells. D, Lymphocyte adherence to HECs in the presence of a nonbinding mAb 3G6 and anti-CD73 mAbs 4G4 and 1E9, which recognize distinct epitopes of the CD73 molecule.

**FIGURE 2.** Lymphocyte pretreatment, but not EC pretreatment, with mAb 4G4 increases lymphocyte binding to EC. Lymphocytes or HECs were separately preincubated with the anti-CD73 mAb 4G4 or with anti-HLA-ABC mAb HB116, and the Ab was washed away before performing the adhesion assay to demonstrate whether the CD73 molecule expressed on PBL or the molecule on EC is important in regulating lymphocyte adhesion. Pretreatment of lymphocytes with 4G4 caused a 2.3-fold increase in lymphocyte binding to EC compared with anti-HLA-ABC mAb HB116, whereas there was only a marginal increase of 23% when the Ab was applied solely to the EC side (Fig. 2; n = 4). Since practically all EC express CD73 on their surface at a high level, it is likely that CD73 expressed on the lymphocytes is involved in the increased lymphocyte binding, and this result suggests that shedding of CD73 from the lymphocyte surface might be essential in regulating lymphocyte adhesion, and it takes place subsequent to ligand binding to the same region recognized by 4G4 mAb.

**CD73 on lymphocytes, but not on endothelium, is responsible for increased adhesion**

Lymphocytes and EC were separately preincubated with the anti-CD73 mAb 4G4 or with anti-HLA-ABC mAb HB116, and the Ab was washed away before performing the adhesion assay to demonstrate whether the CD73 molecule expressed on PBL or the molecule on EC is important in regulating lymphocyte adhesion. Pretreatment of lymphocytes with 4G4 caused a 2.3-fold increase in lymphocyte binding to EC compared with anti-HLA-ABC mAb HB116, whereas there was only a marginal increase of 23% when the Ab was applied solely to the EC side (Fig. 2; n = 4). Since practically all EC express CD73 on their surface at a high level, it is likely that CD73 expressed on the lymphocytes is involved in the increased lymphocyte binding, and this result suggests that shedding of CD73 from the lymphocyte surface might be essential in regulating lymphocyte adhesion, and it takes place subsequent to ligand binding to the same region recognized by 4G4 mAb.
We used a mAb against epitope 24 to detect possible affinity. Epitope 24 serves as a reporter of a high-affinity state of LFA-1. LFA-1 in physiological conditions does not result in a high-affinity state of Fc receptors. Binding of mAb to the CD73 molecule instead of nonspecific binding confirm that the effect of 4G4 mAb is caused by specific binding (B). The results obtained were comparable and are shown here together as the mean ± SEM.

is possible that the 23% increase is due to Abs detaching from the EC during the adhesion assay and becoming free to affect lymphocytes.

LFA-1 mediates increased lymphocyte binding to endothelium

If anti-CD18 mAb HB203, which recognizes the β-chain of the leukocyte integrin LFA-1 (αLβ2), is applied to the adhesion assay simultaneously with the 4G4 mAb, the increase caused by the 4G4 mAb is abolished, and, instead, the adhesion level is 25% lower than that in the presence of the irrelevant control Ab 3G6 (Fig. 3; p = 0.0116). If 4G4 mAb is applied simultaneously with 3G6, the adhesion level is practically unaltered compared with that using 4G4 mAb alone (Fig. 3; n = 5). When either 4G4 mAb alone or both 4G4 mAb and 3G6 mAb were applied to the adhesion assay, there was an ~60% increase in the level of adhesion compared with that using irrelevant mAb 3G6 (Fig. 3; p = 0.0206 and p = 0.01986, respectively). This result implies that the CD73 molecule is probably functioning in concert with LFA-1 to regulate lymphocyte adhesion. The experiments were repeated with slight modification. In these experiments lymphocytes were pretreated with human γ-globulin to block nonspecific binding of Abs to lymphocyte Fc receptors, and the results were practically identical with those described above. The experiments with γ-globulin pretreatment confirm that the effect of 4G4 mAb is caused by specific binding of mAb to the CD73 molecule instead of nonspecific binding to Fc receptors.

CD73 engagement does not result in a high-affinity state of LFA-1 in physiological conditions

Epitope 24 serves as a reporter of a high-affinity state of LFA-1. We used a mAb against epitope 24 to detect possible affinity changes in LFA-1 subsequent to engagement of CD73. Anti-CD73 treatment did not result in any detectable level of epitope 24 expression when measured using immunofluorescence staining followed by flow cytometric analysis in physiological conditions, i.e., in the medium containing Ca2+/Mg2+ (Fig. 4). In contrast, in Ca2+/Mg2+-free medium, weak expression of epitope 24 was observed. Mn2+ treatment caused high expression of epitope 24. Mean fluorescence intensities are shown in the upper corners of the histograms. The experiment was performed three times with similar results. The x-axis is the fluorescence intensity on a log scale, and the y-axis is the relative number of cells.

CD73 engagement causes clustering of LFA-1

Next, we wanted to determine whether engagement of CD73 leads to increased avidity of LFA-1 that can be recognized as altered distribution of LFA-1 on the cell surface. Possible changes in distribution were studied using confocal microscopy and Abs against CD18 and CD11a. Comparable results were obtained with both Abs. Results with anti-CD18 are shown in Fig. 5. Subsequent to engagement of CD73 with mAb 4G4, about 15–20% (depending on the experiment) of the cells clustered their LFA-1, which was seen as intense spot-like staining. With the instrumental settings used the clusters were seen as yellow spots, and cells containing those spots were counted as positive (Fig. 5A). The percentage of the cells with clustered LFA-1 was compatible with the number of CD73-positive cells in the samples, although due to shedding of CD73 after engagement, direct determination of CD73-positive cells is impossible. Ionomycin-induced clustering of LFA-1 has been shown to involve a calpain-like enzyme, a...
multifunctional cytosolic protease that is activated by Ca\(^{2+}\) (28, 30–32). To investigate whether CD73-triggered clustering of LFA-1 is also dependent on calpain activity, we treated duplicate samples with a membrane-permeable calpain inhibitor, calpeptin. Calpeptin treatment efficiently blocked the cluster formation in CD73-triggered cells, as cells with bright yellow spots were not seen (Fig. 5B). Ionomycin treatment caused efficient clustering in the majority of cells, and it was inhibitable with calpeptin (Fig. 5C and D). No LFA-1 clusters were detected in samples triggered with mAb 3G6 (Fig. 5F). To quantitate the density of the brightest spots, the instrumental settings were applied in which signals from 4G4- or ionomycin-treated, calpain-treated cells were used as a cutoff. Subsequent to 4G4 or ionomycin treatment, CD18-positive cells (without calpain treatment) displayed 8–15 intense spots of variable sizes per cell. Clustering of LFA-1 was a specific consequence of CD73 engagement, because engagement of CD73 did not induce changes in the cellular distribution of CD8 (data not shown). These results clearly show that CD73 engagement causes clustering of LFA-1, and a calpain-like enzyme has a central role in this clustering.

**FIGURE 5.** Engagement of CD73 causes clustering of LFA-1. A, PBL were triggered with 4G4 mAb against CD73, and clustering of LFA-1 was detected with FITC-conjugated anti-CD18 Ab. The highest fluorescence intensity is shown as yellow spots and indicates the most intense clustering (arrows). B, The most intense clustering (bright yellow spots) is not seen after calpeptin treatment. C, Ionomycin treatment results in cluster formation of LFA-1 (two intense clusters are pointed out by arrows). D, Ionomycin-induced clustering is inhibitable by calpeptin. E, Staining with a negative FITC-conjugated control Ab after mAb 4G4 treatment; the only signal above the background comes from debris (arrowhead). F, No clustering of LFA-1 (detection with anti-CD18) is visible after pretreatment with an irrelevant class-matched control Ab. The experiment was repeated twice with similar results. Scale bar, 10 μm.

**Discussion**

Our previous results revealed that engagement of lymphocyte CD73 results in shedding of CD73 and in tyrosine phosphorylation and dephosphorylation of intracellular protein substrates (25). This observation led us to study the functional consequences of CD73 engagement. In this work, we report that engagement of lymphocyte CD73, but not endothelial CD73, markedly enhances lymphocyte binding to endothelial cells and that the increased binding is mediated by LFA-1. Moreover, we show that engagement of CD73 does not induce a high-affinity state for LFA-1 receptors in physiological conditions. Instead, CD73 engagement alters the avidity of LFA-1 by calpain-dependent cluster formation, and, more importantly, the clustering is an important adhesion-enhancing event. Thus, we have been able to elucidate the mode of action of CD73 in the multistep adhesion cascade.

Activation of integrins is a critical step during lymphocyte extravasation from the blood into the tissues. The dogma has been that certain chemokines presented by proteoglycan molecules on the endothelial cell surface bind to their receptors on the lymphocyte surface, and this interaction leads to integrin activation (inside-out signaling) (33). Our results clearly show that integrin activation to a high-avidity state can also be achieved by means other than chemokine engagement.
than chemokines and their receptors. Most important, the avidity change in LFA-1 subsequent to engagement of CD73 results in markedly enhanced binding of lymphocytes to endothelial cells. Although LFA-1 activation can be achieved by engagement several lymphocyte surface molecules, the readout in the previous studies has been homotypic lymphocyte aggregation or binding to isolated molecules. Whether these signaling events are also operative in lymphocyte binding to endothelial cells has remained open (8–14). An exception has been engagement via CD31, which has been shown to enhance LFA-1-mediated binding of lymphokine-activated killer cells to endothelium (17).

CD73 is preferentially expressed on CD8-positive T cells and B cells, whereas only a minor population of CD4-positive T cells expresses CD73 (18). Together, these cells normally form about 15% of PBL (maximally 25% in certain individuals). In practice, it means that LFA-1 activation via engagement of CD73 can only take place among this CD73-positive population, facilitating its adherence to vascular endothelium. An increase in the percentage of bound lymphocytes of all input lymphocytes subsequent to CD73 engagement in our binding experiments reflects the size of the CD73-positive cell population. To date, most studies concerning molecules involved in lymphocyte binding to endothelial cells have measured the binding of either unseparated lymphocytes or well-defined lymphocyte populations such as CD4-, CD8-, or CD19-positive cells, and of the studied molecules only the contribution of vascular adhesion protein-1 seems to be subtype restricted, i.e., it preferentially mediates adherence of CD8-positive cells to the endothelium (34). Our present results suggest that trafficking of lymphocytes in the body is not regulated only by the subtype-specific manner that is based on the conventional phenotype definition of the lymphocyte subpopulations. Instead, lymphocyte migration into the tissues is controlled by molecules defining new subpopulations, the members of which may belong to helper and cytotoxic T cells as well as to B cells. We can speculate that CD73 offers an advantage for efficient entrance into the tissues to those cells that express this molecule, and most likely, CD73-negative cell populations have comparable systems to fulfill the same task.

We envision that engagement of CD73 using 4G4 Ab mimics the binding of a natural endothelial cell ligand to lymphocyte CD73 that is operative in vivo conditions. In contrast, we have shown that although only 15% of the blood lymphocytes are positive for CD73, practically all lymphocytes infiltrating skin inflammation are CD73 positive (35). This is in striking contrast with the percentage of CD73-positive cells at other sites of inflammation, where only minor populations are CD73 positive. These findings suggest that vascular endothelium in inflamed skin is a preferential expression site of a ligand for CD73.

Many studies elucidating activation of LFA-1 have used Mn2+, which induces the high-avidity state to LFA-1 receptors, and mAb 24 has served as an excellent reporter of this high-avidity state (28, 36). The induction of the high-avidity state and the appearance of epitope 24 do not require intracellular signaling and cytoskeletal changes (28). In contrast, inducers such as ionomycin and thapsigargin that increase the avidity of LFA-1 by clustering the molecule reorganize of the cytoskeleton (28). Moreover, calpain, whose activity is regulated by Ca2+, plays a fundamental role in this process. It is speculated that the protease activity of calpain releases LFA-1 from its cytoskeletal anchorage and allows clustering of LFA-1 (28). Since we demonstrated that CD73-dependent clustering of LFA-1 is inhibitable by calpeptin, this strongly supports the conclusion that calpain is also involved in CD73-induced clustering of LFA-1. This is in complete concor-
dance with the observations that engagement of CD73 induces the rapid Ca2+ mobilization (19) needed for calpain activation.

As a GPI-linked molecule, CD73 can move rather freely on the cell surface and form clusters with other cell surface molecules, most likely in lipid rafts. Lipid rafts are preformed molecules enriched in signal transduction molecules, actin, and actin-binding proteins that also harbor GPI-linked molecules (37, 38). Interestingly, certain GPI-linked proteins have been shown to clustering with β2 integrins (39, 40), and engagement of the urokinase receptor increases β2 integrin-mediated binding of neutrophils and monocytes to endothelium (41). Thus, it is highly likely that CD73 has the same properties on lymphocytes. It would allow close microenvironmental localization of CD73 and LFA-1, and this close proximity may further facilitate the signaling events triggered via CD73. In contrast, endothelial CD73 may have quite different properties, because the molecular microenvironment is distinct from that on lymphocytes. Our previous results using CD73-transfected COS cells may reflect this fact, because CD73 on this artificial ICAM-1-negative host cell line increased lymphocyte adherence that was inhibitable with anti-CD73 Ab (21). Moreover, in the present work, engagement of endothelial CD73 did not cause any significant increase in lymphocyte binding compared with the engagement of lymphocyte CD73.

Based on the results of this work, we can conclude that CD73 functions at the activation step in the multistep adhesion cascade. In a broader sense, our findings indicate that a GPI-linked cell surface molecule can function as a target and induce an activation state to LFA-1, resulting in enhanced lymphocyte binding to endothelial cells. They further suggest that engagement of a CD73-type molecule may bypass the need for a chemokine, at least in certain circumstances. Moreover, in vivo clustering may be a more common way to enhance the function of LFA-1 than induction of the high-avidity state.

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