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Colon Cancer Cell Adhesion to Endothelial E-Selectin Inhibits Detachment of Endothelial Cells through Activation of β_1-Integrin

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Previous studies have implicated a role for E-selectin in carcinoma cell adhesion to vascular endothelium. We examined the role of colon cancer cell adhesion to vascular endothelium via E-selectin using adenoviral vector-mediated transfection in human umbilical vein endothelial cells (HUVECs). We found that the amount of HUVEC detachment from the gelatin matrix 24 h after LS-180 cell adhesion was inhibited only when the HUVECs were transduced with wild-type E-selectin, but not with a cytoplasmic domain truncated mutant E-selectin or the control Lac-Z vector. We also found that the adhesion of LS-180 cells to wild-type E-selectin transduced HUVEC-induced activation of β_1-integrin receptors without affecting MMP activity. These results indicate that colon cancer cell adhesion via E-selectin inhibits HUVEC detachment from the monolayer, at least in part by modulating β_1-integrin activity in HUVECs. In addition, they indicate the importance of the cytoplasmic domain of E-selectin with this phenomenon.

Key Words: **colon cancer; metastasis; E-selectin; β_1-integrin; endothelial cells.**

The metastatic cascade is a complex series of processes that includes intravasation of tumor cells, transport by circulation, adhesive interaction with endothelial cells, extravasation, colonization, and angiogenesis of the target organ (1). The interaction of tumor cells with vascular endothelium precedes their extravasation into target organs (1).

It is known that tumor cells roll and arrest on vascular endothelium under physiological flow conditions by interaction between E-selectin and cancer glycoprotein (2). Previous study has implicated that E-selectin plays an important role in carcinoma cell adhesion to endothelium, thus, some interaction between E-selectin and cancer cells is thought to be required for tumor extravasation during metastasis (3). Using genetically engineered mice that over express E-selectin, significantly more metastatic lesion formation was observed compared to control mice when colon cancer cells were intravenously injected (4). Metastasis was inhibited by administration of an anti-E-selectin mAb (5). Those studies have indicated a critical role for E-selectin during the metastatic process.

Confluent endothelial cells in culture begin to detach or be shed from the monolayer while maintaining monolayer integrity. However, the adhesion of cancer cells or a subset of T lymphocytes has been reported to affect the number of detached endothelial cells (6, 7). In the current study, using adenoviral vector-mediated transfection in HUVECs of a wild-type E-selectin (WT-E), a tail-less mutant E-selectin (ΔC-E), and a control β-galactosidase (Lac-Z), we examined the potential role of E-selectin during cancer cell adhesion to vascular endothelium.

MATERIALS AND METHODS

Reagents. Two anti-β_1-integrin antibodies, HUTS-21 (purchased from PharMingen, San Diego, CA), and 7B4R (purchased from Santa Cruz Biotechnology, Santa Cruz, CA) were used in this study. Fluorescein-conjugated goat anti-mouse IgG was purchased from Caltag (Burlingame, CA). Matrix metalloproteinase, *o*-phenathroline, aprotinin were obtained from Sigma (St. Louis, MO), and TIMP-1 (tissue inhibitor of metalloproteinase-1) was purchased from Fuji Chemicals (Toyama, Japan). 5-Chloromethylfluorescein diacetate (Cell-tracker) was obtained from Molecular Probes (Eugene, OR).

Cell culture. LS-180, a human colon adenocarcinoma cell line, was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and grown in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 20 mM L-glutamine

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(LS-180 medium). HUVECs were isolated and cultured as previously described (8). The primary culture was serially passaged and maintained in RPMI 1640 supplemented with 20% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 μg/ml endothelial cell growth factor, and 50 μg/ml porcine intestinal heparin (HUVEC medium). For experimental purposes, HUVECs subcultured to passage 2 or 3 were used in this study.

Recombinant adenovirus. Three replication-defective recombinant type 5 adenoviruses were used in these studies: AdRSV-WT-E-sel, AdRSV-ΔC-E-sel, and AdRSV-LacZ. AdRSV β gal was constructed as described previously (9). Both AdRSV-WT-E-sel and AdRSV-ΔC-E-sel use the pJM17 backbone, contain E1/E3 deletions. HUVECs were infected with either of these viruses at m.o.i. of 100 seventy-two hours before the experiments as described previously (10).

LS-180 cell adhesion assay. HUVECs, harvested from monolayers, were seeded on 0.1% gelatin-coated 24-well culture plate (2.0×10^5 cells/well) and maintained in HUVEC medium for 4 days to allow formation of a confluent monolayer. LS-180 cells were briefly trypsinized, washed once in LS-180 medium to obtain a single-cell suspension, and allowed to recover in assay medium at 37°C for 30 min. LS-180 cells were then labeled with Cell-Tracker, essentially as previously reported (11). Adhesion assay was carried out at 37°C in a 5% CO_2 atmosphere for various periods of time. After removing non-adherent LS-180 cells, the fluorescent activity of the adherent LS-180 cells in each individual well was measured by a fluorescent plate reader with a 485/20-nm extension and 530/30-nm emission (Perseptive Biosystems, Framingham, MA).

Endothelial detachment experiments. HUVECs (2.0×10^5 cells/well) were plated onto a gelatin-coated 24-well culture plate. The HUVEC monolayer was labeled with Cell-Tracker for detachment quantitation. Single-cell suspension of LS-180 cells (6.4×10^4 cells/well) were added to endothelial monolayers in the assay medium and incubated at 37°C for 24 h. For quantitation of HUVEC detachment, HUVECs released into the supernatant were collected by centrifugation and their fluorescent activity was measured as described above.

Immunoprecipitation and Western blotting of cellular proteins. HUVECs (8.0×10^6 cells/well) were plated onto a gelatin-coated 15-cm culture plate, and LS-180 cells (2.6×10^6 cells/well) were added. After 24 h of incubation, the co-culture of LS-180 cells and HUVECs were rinsed once with ice-cold PBS and then scraped off from the plate in the presence of 100 μl of lysis buffer (20 mM Tris, 1% Tx-100, 1 mM PMSF, 20 μg/ml aprotinin, 10 μg/ml leupeptin). Total cell lysates were subjected to Western blotting as described below. In some experiments, the lysates were precleared with 50 μl of cyanogen bromide-activated Sepharose beads for 1 h at 4°C, and were incubated with 2–3 μg of mAb 7B4R directed against human β_1-integrin for 1 h at 4°C. Fifty micrograms of goat anti-mouse IgG-coupled Sepharose beads (ICN Pharmaceuticals, Aurora, OH) was then added to the mixture and incubated for 1 h at 4°C. The beads were washed twice each with three different washing buffers (600 mM NaCl, 0.1 M Tris–HCl, and 5 mM EDTA, pH 7.4 containing 1% Triton X-100; 300 mM NaCl, 0.1 M Tris–HCl, and 5 mM EDTA, pH 7.4; 150 mM NaCl, 0.1 M Tris–HCl, and 5 mM EDTA, pH 7.4). The immunoprecipitated proteins were eluted from the beads by boiling for 5 min in 30 μl of 0.5 M Tris–HCl (pH 6.8) containing 0.4% SDS.

Equal amounts of the samples were separated by SDS–PAGE under reducing conditions and transferred to PVDF membranes (Millipore, Bedford, MA), which were then blocked with 5% dry milk in TBS-T (20 mM Tris, 137 mM $NaCl_2$, 0.1% Tween 20, pH 7.6) for 90 min, and followed by incubation with the anti-β_1-integrin mAb HUTS-21 (1:1000 diluted in blocking buffer) for 1 h. After three washes with TBS-T, the membranes were incubated with HRP-conjugated polyclonal goat anti-mouse (or anti-rabbit or anti-rat) Ab (1:3000 diluted in blocking buffer) for an additional hour at room temperature, and washed three times in TBS-T. The labeled proteins were visualized using an enhanced chemiluminescence kit (Amersham, Arlington Heights, IL), following the manufacturer's protocol.

Statistics. Data are presented as means ± standard deviation (SD) as indicated. Two-tailed Student *t* tests were performed with Microsoft Excel. *P* values represent the results of these *t* tests and *P* values of <0.05 were considered statistically significant.

RESULTS

LS-180 Adhesion to HUVECs Transduced by E-Selectin under Static Assay Conditions

To investigate E-selectin-dependent mechanisms in colon cancer adhesion to vascular endothelium, we utilized two recombinant adenoviral vectors: one containing the entire coding translated region of E-selectin cDNA, AdRSV-WT-E, and the other containing a mutant form of E-selectin lacking the cytoplasmic domain, AdRSV-ΔC-E (12). As previously reported, both of these adenovirus constructs exhibit comparable levels of immunoreactive cell surface E-selectin expression, as confirmed by fluorescence immunoassay (10).

Adhesion of colon cancer cells to E-selectin was examined using human colon cancer derived LS-180 cells (13). LS-180 cell adhesion to both WT-E and ΔC-E transduced HUVECs were studied under static assay conditions. As shown in Fig. 1, both AdRSV-WT-E and AdRSV-ΔC-E transduced HUVECs exhibited significantly more adhesion with LS-180 after 10 min of incubation, as compared to HUVECs transduced with the control Lac-Z adenovirus (Fig. 1A). The amount of LS-180 adhesion was not significantly different between AdRSV-WT-E and AdRSV-ΔC-E transduced HUVECs, which was similar to our previous findings using HL-60 cells (12). When the co-incubation was extended to 24 h, however, the numbers of LS-180 cells found adhered to inactivated and Lac-Z transduced HUVEC were markedly increased, and comparable to the levels observed with E-selectin (both WT-E and ΔC-E) transduced HUVECs (Fig. 1B).

Adhesion of LS-180 Cells Inhibits HUVEC Detachment from Gelatin Matrix

We then observed the effect of LS-180 cells on the integrity of the HUVEC monolayer during their adhesive interactions. It was previously shown that adhesion of cancer cells to vascular endothelium induces detachment of endothelial cells from the monolayer (6). To examine the detachment of HUVECs from the monolayer, culture supernatants containing detached HUVECs were collected from each culture well after 24 h of coincubation with LS-180 cells. Light microscopic analysis of the supernatant (Fig. 2A) revealed numerous cells detached in the wells that contained HUVECs transduced with control AdRSV-Lac-Z after LS-180 adhesion (see Fig. 2A, f). If contrast, there were significantly fewer detached cells in the wells contain-

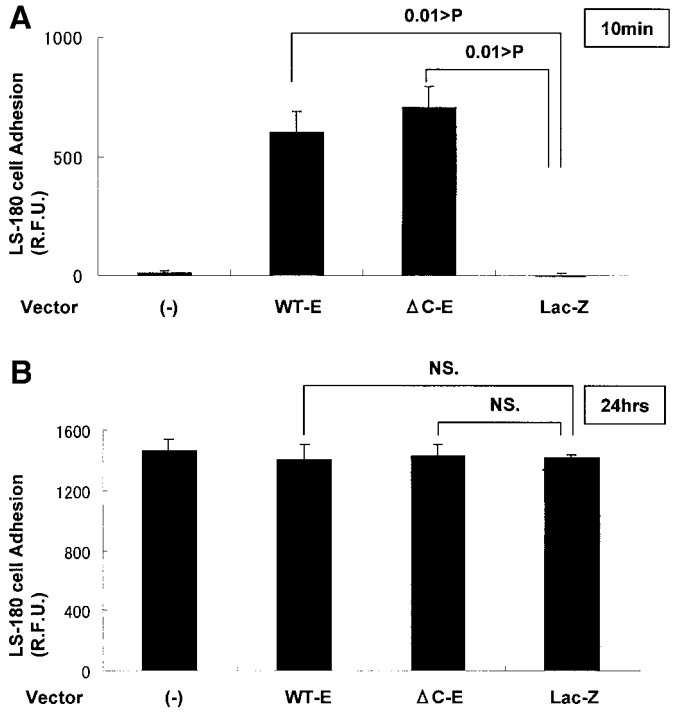


FIG. 1. LS-180 cells, a human colon cancer cell line, were added (6.4×10^4/well) to AdRSV-WT-E-selectin (WT-E), AdRSV-ΔC-E-selectin (ΔC-E), or control AdRSV-Lac-Z (Lac-Z) transduced HUVECs (100 m.o.i.), and then adhesion assays were carried out as described under Materials and Methods. The number of adhered LS-180 cells after 10 min (A) or 24 h (B) was expressed as relative fluorescent units (RFU) per well. Data are representative of four independent experiments.

ing HUVECs transduced with AdRSV-WT-E after LS-180 adhesion (see Fig. 2A, d). Interestingly, this reduced cell detachment was not observed in wells containing HUVECs transduced with the cytoplasmic domain deleted AdRSV-ΔC-E (see Fig. 2A, e). No significant cell detachment was observed after 10 min of LS-180 adhesion (data not shown). These phenomena was not observed when HUVECs were activated with IL-1β to induce endogenous E-selectin (data not shown). To more precisely determine the number of detached HUVECs (be excluding any LS-180 cells in the supernatant), HUVEC monolayers were fluorescently labeled prior to LS-180 cell adhesion and the number of detached HUVECs was estimated by measuring their fluorescent intensity. As shown in Fig. 2B, HUVEC detachment from the monolayer after LS-180 adhesion was significantly reduced when transduced with AdRSV-WT-E ($37.5 \pm 8.2\%$ of the control, $P < 0.002$), but not with AdRSV-ΔC-E ($94.3 \pm 19.2\%$ of the control, $P = 0.68$) or AdRSV-Lac-Z ($97.7 \pm 9.7\%$ of the control, $P = 0.78$). Further, detachment of HUVECs occurred in a time-dependent manner, as shown in Fig. 2C. As previously reported (14), HUVECs underwent apoptosis when detached from the monolayer. To determine whether detached HUVECs were undergoing apoptosis, we examined the detached cells for their binding activity with PE-conjugated annexin V by measuring fluorescence intensity. About 90% of the detached cells were positive for annexin V regardless of adenovirus treatment, though the numbers of detached cells were significantly different among the various conditions described above (data not shown).

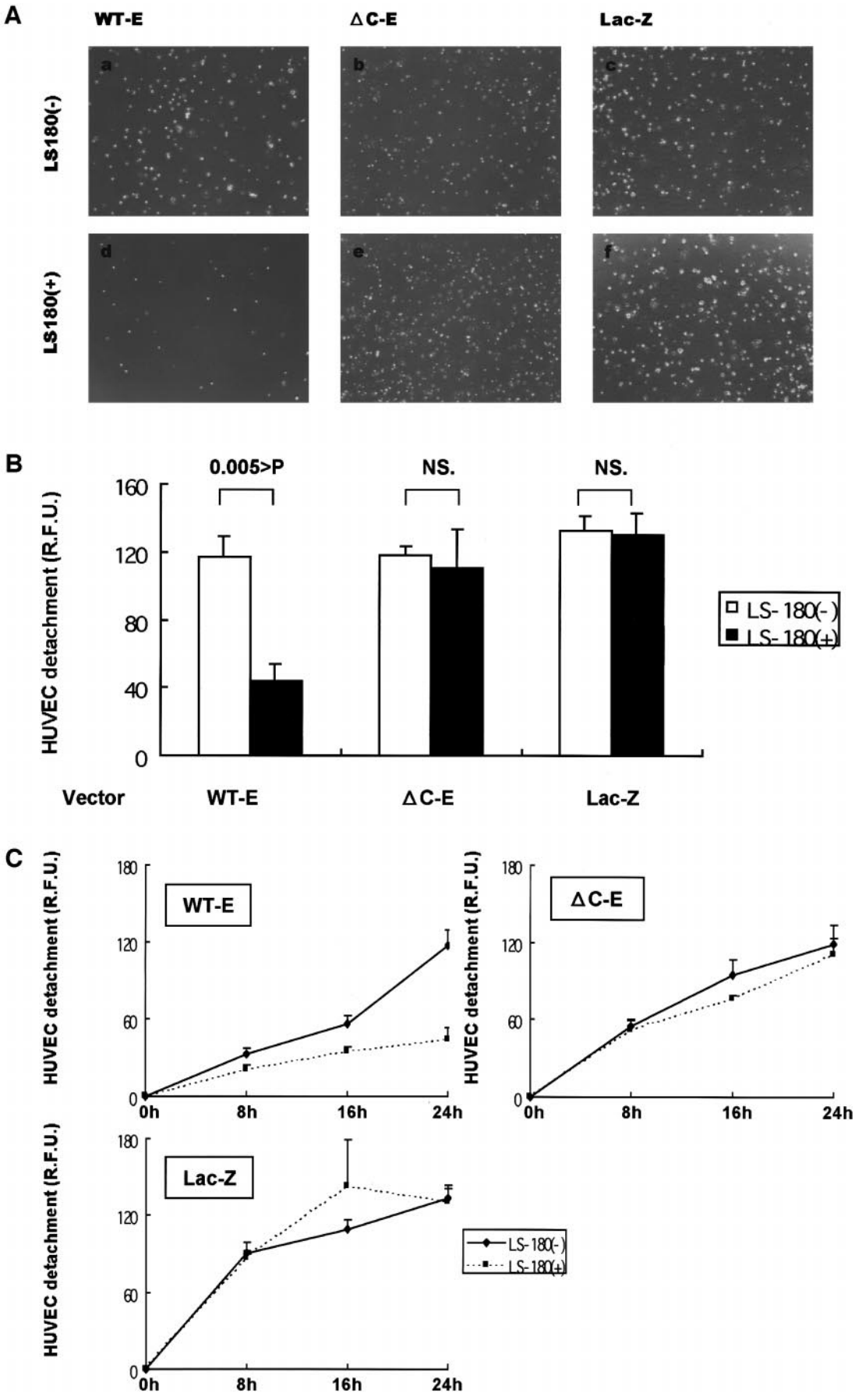
LS-180 Cell Adhesion Activates β_1-Integrin in AdRSV-WT-E Transduced HUVECs

To study the mechanism for reduced HUVEC detachment after WT-E- but not ΔC-E-dependent LS-180 adhesion, the expression level of β_1-integrin, one of the adhesion molecules responsible for endothelial anchoring to the extracellular matrix, was examined by Western blotting analysis after LS-180 adhesion. Two different anti-β_1-integrin mAbs, HUTS-21, which recognizes the activation-dependent epitope of β_1-integrin (15), and 4B7R, which reacts with both the active and resting forms of β_1-integrin were utilized.

As shown in Fig. 3A, mAb HUTS-21 recognized a significantly greater amount of immunoreactive β_1-integrin from 4B7R immunoprecipitate prepared from HUVECs transduced with WT-E only after LS-180 adhesion. Judged from Western blotting with mAb 4B7R, the total amount of β_1-integrins, was not changed in the HUVECs under any of these conditions.

To further investigate the role of β_1-integrin during this process, we plated HUVECs onto a gelatin matrix in the presence of the anti-β_1-integrin mAb to block the anchoring of HUVEC via β_1-integrins. After 72 h, the integrity of the monolayer was examined by a phase-contrast microscope prior to the adhesion assay (data not shown). LS-180 cells were then added to the

FIG. 2. (A) Microscopic images of detached cells present in culture supernatants recovered from AdRSV-WT-E-selectin (WT-E) [a, d], AdRSV-ΔC-E-selectin (ΔC-E) [b, e], and AdRSV-Lac-Z (Lac-Z) [c, f] transduced HUVECs after 24 h of coincubation with [d, e, f] or without [a, b, c] LS-180 cells (magnification ×40). (B) HUVECs transduced with AdRSV-WT-E-selectin (WT-E), AdRSV-ΔC-E-selectin (ΔC-E), or AdRSV-Lac-Z (Lac-Z) were labeled with Cell-Tracker fluorescent dye and then cultured in the absence [LS-180(−)] or presence [LS-180(+)] of LS-180 cells (6.4×10^4 cells/well) for 24 h. Data are representative of four independent experiments. Error bars indicate standard deviations for triplicate cultures. (C) Time course of HUVEC detachment induced by LS-180 adhesion to monolayers transduced with AdRSV-WT-E-selectin (WT-E), AdRSV-ΔC-E-selectin (ΔC-E), or AdRSV-Lac-Z (Lac-Z). Fluorescently labeled HUVECs were coincubated with (hatched line) or without (black line) LS-180 cells for the indicated times prior to the measurement of detached HUVECs in the supernatant. Data are representative of three independent experiments. Error bars indicate standard deviations for triplicate cultures.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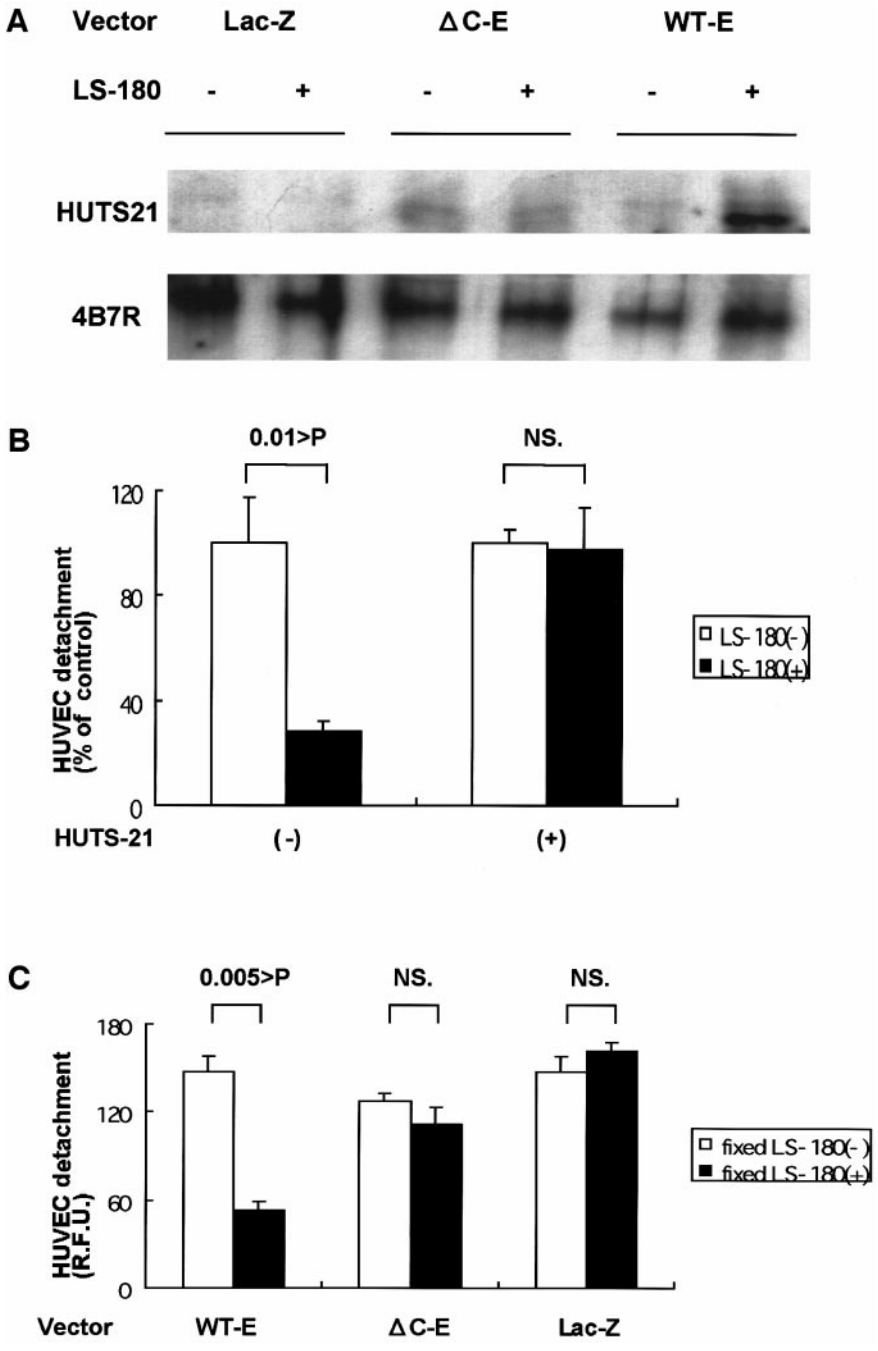

FIG. 3. (A) HUVECs transduced with either WT-E-selectin (WT-E), ΔC-E-selectin (ΔC-E), or Lac-Z (Lac-Z) adenoviral constructs coincubated in the presence (+) or absence (−) of LS-180 cells for 24 h. Total cell lysates recovered from each culture were subjected to immunoprecipitation (IP) using β_1-integrin monoclonal Ab (4B7R). Western blotting was carried out using 4B7R and HUTS-21. (B) HUVEC detachment was prevented by pretreatment of HUVECs with anti-β_1-integrin HUTS-21. HUVECs transduced with AdRSV-WT-E were plated onto a gelatin matrix in the presence (+) or absence (−) of activating anti-β_1-integrin HUTS-21 prior to LS-180 cell adhesion, and then were cultured in the presence [LS-180(+)] or absence [LS-180(−)] of LS-180 cells, (6.4×10^4 cells/well) for 24 h. HUVEC detachment was measured as described previously. Data are representative of three independent experiments. Error bars indicate standard deviations for triplicate cultures. (C) HUVECs transduced with AdRSV-WT-E-selectin (WT-E), AdRSV-ΔC-E-selectin (ΔC-E), or AdRSV-Lac-Z (Lac-Z) were labeled with Cell-Tracker fluorescent dye and then cultured in the presence [fixed LS-180(+)] or absence [fixed LS-180(−)] of fixed LS-180 cells (6.4×10^4 cells/well) for 24 h. Data are representative of four independent experiments. Error bars indicate standard deviations for triplicate cultures.

HUVEC monolayer that was anchored independent of β_1-integrin in the continuous presence of HUTS-21. As shown in Fig. 3B, HUVEC detachment was significantly increased in the presence of this mAb ($P < 0.005$), even from the monolayers transduced with WT-E.

Adhesion of Fixed LS-180 Cells Inhibits HUVEC Detachment

To examine the possible effect of β_1-integrins expressed in LS-180 cells in this process, we utilized LS-180 cells fixed with 2% paraformaldehyde (4°C, 20 min) and conducted the detachment assay described above. As shown in Fig. 3C, HUVEC detachment from the monolayer after fixed-LS-180 adhesion was significantly reduced when HUVECs were transduced with AdRSV-WT-E, but not with AdRSV-ΔC-E or AdRSV-Lac-Z, which was similar to results of the experiments using live LS-180 cells.

Matrix Metalloproteinase (MMP) Was Not Involved in HUVEC Detachment

Since transcriptional activation of MMP has been reported in both HUVECs and lymphocytes upon activation, we examined the possibility that contact between LS-180 and HUVECs might induce matrix degrading MMPs to mediate HUVEC detachment (16). HUVEC detachment was not inhibited by *o*-phenathroline (a broadly active MMP inhibitor), or TIMP-1 (tissue inhibitor of metalloproteinase-1) (data not shown). We also verified that aprotinin, a serine protease inhibitor capable of degrading extracellular matrix, failed to inhibit HUVEC detachment (data not shown). These data suggest that matrix MMP activity does not account for LS-180-stimulated HUVEC release from the matrix.

DISCUSSION

The selectin family of adhesion molecules mediates the interaction of circulating leukocytes with vascular endothelium in response to inflammatory stimuli (17). E-selectin recognizes the sialyl-Lewis X determinant found on the surface of leukocytes as well as in some tumor cell lines (17). It has also been shown that E-selectin plays a role in colon carcinoma cell adhesion to activated endothelial cells (18). This E-selectin dependent cancer cell adhesion to vascular endothelium may be crucial in the process of cancer metastasis (19).

The expression of E-selectin was previously regarded to occur exclusively in vascular endothelium. However, a recent study reported evidence of E-selectin expression without an obvious inflammatory stimulus (19), while another report suggested that an alternative isoform of E-selectin carrying a different length of 3′ flanking region exhibited prolonged expression (20). More recently, nonendothelial cells such as T lymphocytes have been reported to be able to express E-selectin under certain conditions (21). These data point to the possibility that the expression pattern of E-selectin could be more complicated than once believed. Notably, in the context of cancer cell adhesion during metastasis, a sustained E-selectin expression may be favorable for cancer cell adherence. Since we were able to express E-selectin for a relatively extended period using a recombinant adenovirus system, we decided to examine LS-180 adhesion to E-selectin transduced HUVECs for up to 24 h.

In the present study, we demonstrated that detachment of HUVECs from the monolayer 24 h after LS-180 adhesion was significantly inhibited when HUVECs were transduced with wild-type E-selectin adenovirus; however, this inhibitory effect was not observed when they were transduced with the control adenovirus. Interestingly, a cytoplasmic domain-deleted (ΔC-E) mutant form of E-selectin failed to inhibit endothelial detachment. These results suggested that the cytoplasmic domain of E-selectin is necessary for the observed reduction of HUVEC detachment.

We also examined the detachment of HUVECs from the monolayer after activation with IL-1β. The number of detachment HUVECs from the cytokine-activated monolayer was not significantly changed between in the presence or absence of LS-180 adhesion (data not shown). Previous studies have demonstrated that endogeneous E-selectin protein expression in HUVECs peaks 4 h after activation and then gradually decreases (8, 22). Thus, no significant E-selectin expression was detected 8 h after stimulation. An extended expression of E-selectin may be necessary for this inhibitory effect on detachment to occur.

Previously, it was shown that the number of tumor-infiltrating lymphocytes in primary carcinomas decreases in parallel with tumor progression and metastasis (23). This supports the hypothesis that local antitumor immunity conferred by the infiltrating lymphocytes may play an important role in controlling progression and metastasis of cancer. Detached endothelial cells in the circulation may activate the immune system to attack cancer cells. Therefore, E-selectin-dependent cancer cell adhesion to endothelium, through a reduction of detached endothelial cells as we observed, may be favorable for cancer metastasis by providing a means to escape detection from the host immune system. Our results indicate the importance of β_1-integrins during detachment of HUVECs. It is known that β_1-integrin plays a critical role in the anchoring of endothelial cells by binding to the extracellular matrix (24). Therefore, a reduction of the binding activity of β_1-integrin would explain the detachment of HUVECs from the matrix. LS-180 adhesion to E-selectin may induce an intracellular signal to activate β_1-integrin, thus preventing their detachment. Since cytoplasmic deletion of the E-selectin mutant failed to inhibit endothelial detachment, the presence of the E-selectin cytoplasmic domain may be required for the E-selectin-dependent signaling pathway. A recent study indicated evidence of an E-selectin-dependent signaling pathway in HUVECs by demon-

strating a dynamic association with the endothelial cytoskeleton (12) and activation of MAPK (25). The present study results further validate the importance of the E-selectin cytoplasmic domain, though elucidation of the details of the cellular mechanism between E-selectin and β_1-integrin will require additional experiments. The activity of β_1-integrins in LS-180 cells may be neglected, since fixed LS-180 cells exhibited a similar inhibitory effect on E-selectin transduced-endothelial detachment. However, a monoclonal Ab against E-selectin as well as magnetic beads coated with anti-E-selectin mAb failed to inhibit endothelial detachment (data not shown). HL-60 cells, a leukocyte cell line that carries the E-selectin ligand, exhibited a similar inhibition of endothelial detachment, though in lower amounts, when adhered to WT-E-selectin transduced HUVECs for 24 h. These results suggest that the mechanisms by which E-selectin activates β_1-integrin may require additional interactions of membrane receptors or adhesion molecules between LS-180 cells and HUVECs. MMP inhibitors failed to inhibit these phenomena, thus implying that MMPs do not play a major role (data not shown).

In conclusion, we found that the E-selectin cytoplasmic domain is required for the inhibition of HUVEC detachment when LS-180 cells were adhered to HUVECs. This finding is the initial evidence of the biological importance of the E-selectin cytoplasmic domain.

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REFERENCES

1. Bohle, A. S., and Kalthoff, H. (1999) Molecular mechanisms of tumor metastasis and angiogenesis. *Langenbecks Arch. Surg.* **384**(2), 133–140. [review]
2. Goetz, D. J., Brandley, B. K., and Hammer, D. A. (1996) An E-selectin-IgG chimera supports sialylated moiety dependent adhesion of colon carcinoma cells under fluid flow. *Ann. Biomed Eng.* **24**(1), 87–98.
3. McEver, R. P. (1997) Selectin–carbohydrate interactions during inflammation and metastasis. *Glycoconj. J.* **14**(5), 585. [review]
4. Biancone, L., Araki, M., Araki, K., Vassalli, P., and Stamenkovic, I. (1996) Redirection of tumor metastasis by expression of E-selectin *in vivo*. *J. Exp. Med.* **183**(2), 581–587.
5. Brodt, P., Fallavollita, L., Bresalier, R. S., Meterissian, S., Norton, C. R., and Wolitzky, B. A. (1997) Liver endothelial E-selectin mediates carcinoma cell adhesion and promotes liver metastasis. *Int. J. Cancer* **71**(4), 612.
6. Kebers, F., Lewalle, J. M., Desreux, J., Munaut, C., Devy, L., Foidart, J. M., and Noel, A. (1998) Induction of endothelial cell apoptosis by solid tumor cells. *Exp. Cell Res.* **240,** 197–205.
7. Phan, C., McMahon, A. W., Nelson, R. C., Elliott, J. F., and Murray, A. G. (1999) Activated lymphocytes promote endothelial cell detachment from matrix: A role for modulation of endothelial cell beta 1 integrin affinity. *J. Immunol.* **163**(8), 4557–4563.
8. Bevilacqua, M. P., Pober, J. S., Wheeler, M. E., Cotran, R. S., and Gimbrone, M. A. (1985) Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J. Clin. Invest.* **76**(5), 2003–2011.
9. Gerszten, R. E., Luscinskas, F. W., Ding, H. T., Dichek, D. A., Stoolman, L. M., Gimbrone, M. A., and Rosenzweig, A. (1996) Adhesion of memory lymphocytes to vascular cell adhesion molecule-1-transduced human vascular endothelial cells under simulated physiological flow conditions *in vitro*. *Circ. Res.* **79**(6), 1205–1215.
10. Merrilees, M. J., Beaumont, B. W., and Scott, L. J. (1995) Fluoroprobe quantification of viable and non-viable cells in human coronary and internal thoracic arteries sampled at autopsy. *Vasc. Res.* **32**(6), 371–377.
11. Yoshida, M., Szente, B. E., Kiely, J. M., Rosenzweig, A., and Gimbrone, M. A. (1998) Phosphorylation of the cytoplasmic domain of E-selectin is regulated during leukocyte–endothelial adhesion. *J. Immunol.* **161**(2), 933–941.
12. Yoshida, M., Westlin, W. F., Wang, N., Ingber, D. E., Rosenzweig, A., Resnick, N., and Gimbrone, M. A. (1996) Leukocyte adhesion to vascular endothelium induces E-selectin linkage to the actin cytoskeleton. *J. Cell Biol.* **133**(2), 445–455.
13. Tom, B. H., Rutzky, L. P., Jakstys, M. M., Oyasu, R., Kaye, C. I., and Kahan, B. D. (1976) Human colonic adenocarcinoma cells. I. Establishment and description of a new line. *In Vitro* **12**(3), 180–191.
14. Levkau, B., Herren, B., Koyama, H., Ross, R., and Raines, E. W. (1998) Caspase-mediated cleavage of focal adhesion kinase of pp125FAK and disassembly of focal adhesions in human endothelial cell apoptosis. *J. Exp. Med.* **187**(4), 579–586.
15. Luque, A., Gomez, M., Puzon, W., Takada, Y., Sanchez-Madrid, F., and Cabanas, C. (1996) Activated conformations of very late activation integrins detected by a group of antibodies (HUTS) specific for a novel regulatory region of the common beta 1 chain. *J. Biol. Chem.* **271**(19), 11067–11075.
16. Russell, J. H., Musil, L., and McCulley, D. E. (1988) Loss of adhesion. A novel and distinct effect of the cytotoxic T lymphocyte–target interaction. *J. Immunol.* **140**(2), 427–432.
17. Kansas, G. S. (1996) Selectins and their ligands: Current concepts and controversies. *Blood* **88**(9), 3259–3287.
18. Krause, T., and Turner, G. A. (1999) Are selectins involved in metastasis? *Clin. Exp. Metastasis* **17**(3), 183. [review]
19. Schweitzer, K. M., Drager, A. M., van der Valk, P., Thijsen, S. F., Zevenbergen, A., Theijsmeijer, A. P., van der Schoot, C. E., and Langenhuijsen, M. M. (1996) Constitutive expression of E-selectin and vascular cell adhesion molecule-1 on endothelial cells of hematopoietic tissues. *Am. J. Pathol.* **148**(1), 165–175.
20. Rood, P. M., Dercksen, M. W., Cazemier, H., Kerst, J. M., Von dem Borne, A. E., Gerritsen, W. R., and van der Schoot, C. E. (2000) E-selectin and very late activation antigen-4 mediate adhesion of hematopoietic progenitor cells to bone marrow endothelium. *Ann. Hematol.* **79**(9), 477–484.
21. Harashima, Si., Horiuchi, T., Hatta, N., Morita, C., Higuchi, M., Sawabe, T., Tsukamoto, H., Tahira, T., Hayashi, K., Fujita, S., and Niho, Y. (2001) Outside-to-inside signal through the membrane TNF-alpha induces E-selectin (CD62E) expression on activated human CD4(+) T cells. *J. Immunol.* **166**(1), 130–136.

22. Bevilacqua, M. P., Stengelin, S., Gimbrone, M. A., and Seed, B. (1989) Endothelial leukocyte adhesion molecule 1: An inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* **243**(4895), 1160–1165.
23. Tada, T., Ohzeki, S., Utsumi, K., Takiuchi, H., Muramatsu, M., Li, X. F., Shimizu, J., Fujiwara, H., and Hamaoka, T. (1991) Transforming growth factor-β-induced inhibition of T cell function. Susceptibility difference in T cells of various phenotypes and functions and its relevance to immunosuppression in the tumor-bearing state. *J. Immunol.* **146**(3), 1077–1082.
24. Ingber, D. (1991) Integrins as mechanochemical transducers. *Curr. Opin. Cell Biol.* **3**(5), 841–848. [review]
25. Hu, Y., Kiely, J. M., Szente, B. E., Rosenzweig, A., and Gimbrone, M. A. (2000) E-selectin-dependent signaling via the mitogen-activated protein kinase pathway in vascular endothelial cells. *J. Immunol.* **165**(4), 2142–2148.