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Very Late Antigen-4–Dependent Adhesion of Lymphocytic Cell Lines to Cultured Human Endothelium

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INTRODUCTION

Lymphocyte adhesion to cultured endothelium has recently been shown to involve two members of the integrin family of cell surface heterodimers; namely, lymphocyte function–associated antigen 1 (LFA-1) and very late antigen-4 (VLA-4) (1,2). Several endothelial cell counterreceptors for these two molecules have also been identified: LFA-1 can bind to intercellular adhesion molecule 1 (ICAM-1) or to ICAM-2 (1,3), whereas VLA-4 can bind to vascular cell adhesion molecule 1 (VCAM-1) (2,4,5).

To determine whether there might exist counterreceptors for VLA-4 other than VCAM-1, we have compared the ability of anti–VLA-4 vs anti–VCAM-1 monoclonal antibodies (MAbs) to block adhesion of human lymphocytic cell lines to stimulated and unstimulated cultured human endothelium. Results reported here suggest that VLA-4–dependent lymphocyte-endothelial interactions may involve inducible counterreceptors distinct from VCAM-1.

MATERIALS AND METHODS

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Clonetics (San Diego, California) and maintained for up to 20 doublings in RPMI 1640 with 20% fetal bovine serum, 100 μg/ml bovine endothelial cell growth supplement (Biomedical Technologies, Stoughton, Massachusetts), 100 μg/ml heparin, 20 mM HEPES (N-2-hydroxyethylpoperazine-N-2-ethanesulfonic acid), 5 mM glutamine, and 50 μg/ml gentamicin. Tissue culture surfaces were pretreated with 1 $\mu g/cm^2$ of human plasma fibronectin in Hank's balanced salt solution. For binding assays, HUVECs were grown to confluence in 96-well tissue culture plates.

Human lymphocytic cell lines were maintained in complete media (i.e., RPMI 1640 with 10% fetal bovine serum, 5 mM glutamine, and 50 μg/ml gentamicin).

Monoclonal Antibodies

The MAbs used were TS1/22 (anti–LFA-1); HP2/1 (anti–VLA-4; a gift of Dr. F. Sanchez-Madrid, Universidad Autonoma de Madrid, Spain); E1/6 (anti–VCAM-1; a gift of Dr. G. E. Rice, Brigham and Women's Hospital, Boston, Massachusetts); and 4B9 (anti–VCAM-1; a gift of Dr. J. Harlan, University of Washington, Seattle, Washington).

HUVEC Stimulation

Recombinant human tumor necrosis factor α (TNFα) (Genzyme, Boston, Massachusetts) was used at 25 ng/ml for 24 hr to stimulate HUVECs in culture.

Adhesion Assay

Lymphoid tumor cells were labeled with the carboxyfluorescein compound BCECF-AM (Molecular Probes Inc., Eugene, Oregon) and resuspended in complete media with 20 mM HEPES. Cells were preincubated with control media or with MAb TS1/22 and/or HP2/1 for 30 min at 37°C. The HUVEC monolayers in 96-well plates were preincubated with MAbs E1/6 or 4B9 for 30 min at 37°C. Without removal of excess MAbs, lymphoid cells (10^5/well) were overlayed on HUVECs and allowed to settle and adhere for 30 min at 37°C. Nonadherent cells were removed by washing five times with complete media and 20 mM HEPES. Percent binding was determined using a fluorescence concentration analyzer (Pandex Laboratories, Inc., Mundelein, Illinois).

Results

A function-blocking anti–VLA-4 MAb, HP2/1, and two blocking anti–VCAM-1 MAbs, E1/6 and 4B9, were compared for their ability to inhibit the adhesion of four lymphocytic cell lines to TNF-stimulated and unstimulated HUVECs. To block any simultaneous interactions between LFA-1 and endothelial ICAM-1 or ICAM-2, cell line binding to HUVECs was assessed in all experiments in the presence of the anti–LFA-1 MAb TS1/22. For the four cell lines examined—T lymphocytic lines Jurkat and SKW3 and B lymphocytic cell lines Raji and Ramos—preincubation with TS1/22 MAb alone blocked adhesion to stimulated or unstimulated HUVECs by <10% compared to preincubation with control media, the control MAb X63, or the anti-CD44 MAb F10442 (Fig. 1). As a control, TS1/22 was observed to block completely the adhesion of the lymphoblastoid cell line JY to TNF-stimulated HUVECs.

Additionally preincubating each of the four cell lines with the anti–VLA-4 MAb HP2/1 significantly blocked adhesion to TNF-stimulated HUVECs (shown in Fig. 1 for one of at least three representative experiments per cell line), as expected from results of a previous study (2). Adhesion of Ramos and Raji cells was blocked by >80%, SKW3 cells by about 70%, and Jurkat cells by 30%. In contrast, preincubation of TNF-stimulated HUVECs with either of two MAb against VCAM-1, a ligand of VLA-4 (2), inhibited cell line adhesion by only a fraction of that observed following preincubation with the anti–VLA-4 MAb (see Fig. 1).

For all cell lines, percent binding following preincubation with anti–VLA-4 MAb *plus* anti–VCAM-1 MAb was the same as that following pretreatment with anti–VLA-4 MAb alone. Neither anti–VLA-4 MAb nor anti–VCAM-1 MAbs affected the basal cell line binding to unstimulated HUVECs, with the possible exception of SKW3 cells.

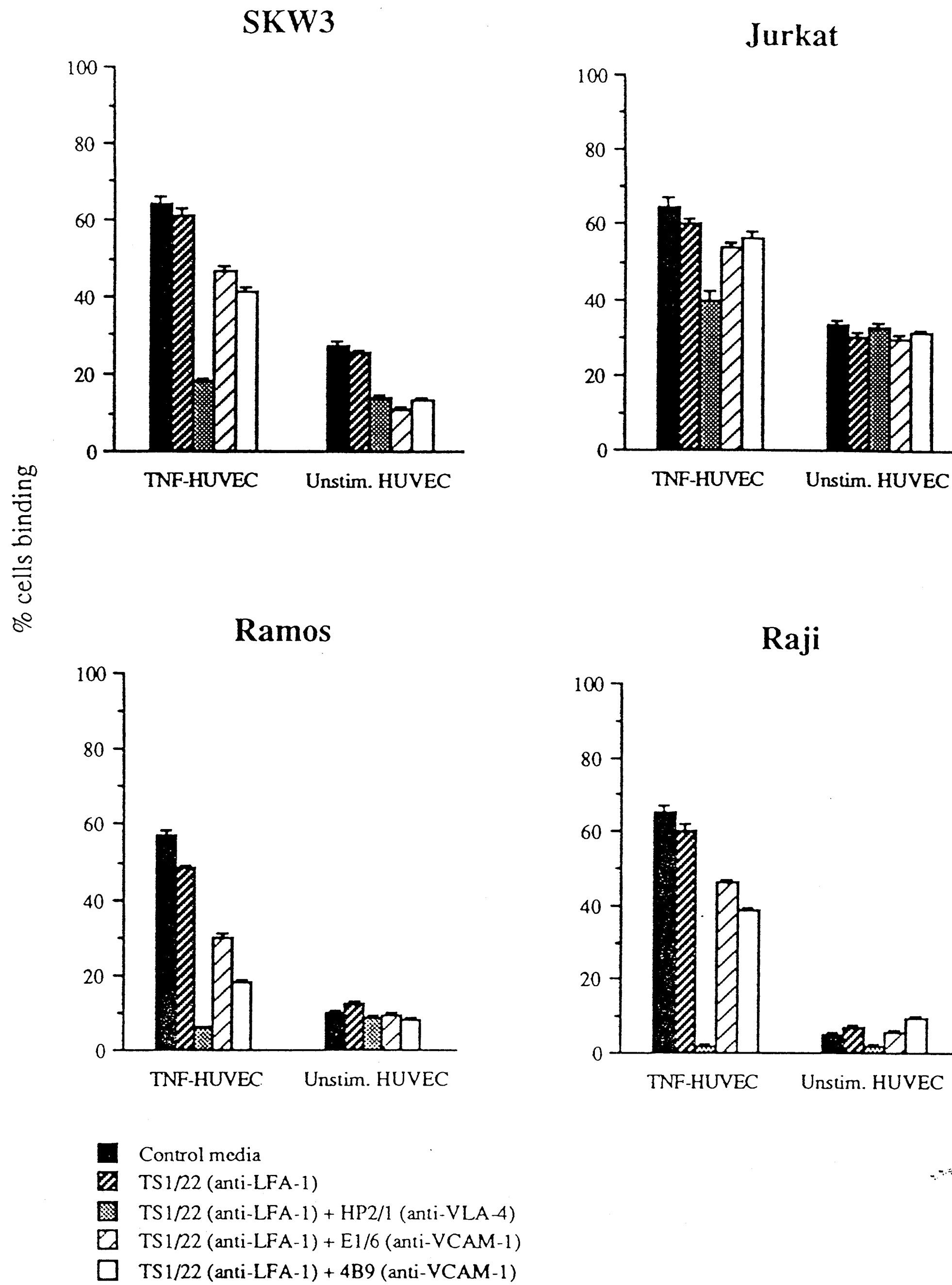

Figure 1 Lymphocytic cell line binding to 24-hr TNF-stimulated or unstimulated human umbilical vein endothelial cells.

DISCUSSION

Preincubation with the anti–VLA-4 MAb HP2/1 blocked adhesion of lymphocytic cell lines to TNF-stimulated HUVECs much greater than preincubation with either of two blocking anti–VCAM-1 MAbs. This observation suggests both a VLA-4–dependent/VCAM-1–independent adhesion pathway as well as a VLA-4–dependent/VCAM-1–dependent pathway mediating lymphocyte-endothelial interactions. Furthermore, comparison of cell line binding to both stimulated and unstimulated HUVECs suggests that the expression of potential VLA-4 counterreceptors distinct from VCAM-1 is cytokine inducible.

Whether such a counterreceptor(s) includes novel inducible surface antigens on endothelial cells and/or extracellular matrix components, such as fibronectin which does interact with VLA-4 (6), is currently being investigated. Preliminary evidence indicates that cell line binding to TNF-stimulated HUVECs is not inhibited further by preincubating HUVECs with an antihuman fibronectin antisera in addition to anti–LFA-1, anti–VLA-4, and anti–VCAM-1 MAbs.

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