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ROLE OF LFA-1 AND VLA-4 IN THE ADHESION OF CLONED NORMAL AND LFA-1 (CD11/CD18)-DEFICIENT T CELLS TO CULTURED ENDOTHELIAL CELLS

Indication for a New Adhesion Pathway

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Patients with the leukocyte adhesion deficiency (LAD) syndrome have a genetic defect in the common β2-chain (CD18) of the leukocyte integrins. This defect can result in the absence of cell surface expression of all three members of the leukocyte integrins. We investigated the capacity of T cell clones obtained from the blood of an LAD patient and of normal T cell clones to adhere to human umbilical vein endothelial cells (EC). Adhesion of the number of LAD T cells to unstimulated EC was approximately half of that of leukocyte function-associated antigen (LFA)-1+ T cells. Stimulation of EC with human rTNF-α resulted in an average 2- and 2.5-fold increase in adhesion of LFA-1+ and LFA-1− cells, respectively. This effect was maximal after 24 h and lasted for 48 to 72 h. The involvement of surface structures known to participate in cell adhesion (integrins, CD44) was tested by blocking studies with mAb directed against these structures. Adhesion of LFA-1+ T cells to unstimulated EC was inhibited (average inhibition of 58%) with mAb to CD11a or CD11b. Considerably less inhibition of adhesion occurred with mAb to CD11a or CD18 (average inhibition, 20%) when LFA-1+ T cells were incubated with rTNF-α-stimulated EC. The adhesion of LFA-1+ T cells to EC stimulated with rTNF-α, but not to unstimulated EC, was inhibited (average inhibition, 56%) by incubation with a mAb directed to very late antigen (VLA)-4 (CDw49d). In contrast to LAD T cell clones and the LFA-1+ T cell line Jurkat, mAb to VLA-4 did not inhibit adhesion of normal LFA-1+ T cell clones to EC, whether or not the EC had been stimulated with rTNF-α. We conclude that the adhesion molecule pair LFA-1/intercellular adhesion molecule (ICAM)-1 plays a major role in the adhesion of LFA-1+ T cell clones derived from normal individuals to unstimulated EC. Adhesion of LFA-1− T cells to TNF-α-stimulated EC is mediated by VLA-4/vascular cell adhesion molecule (VCAM)-1 interactions. Since we were unable to reduce significantly the adhesion of cultured normal LFA-1+ T cells to 24 h with TNF-α-stimulated endothelium with antibodies that block LFA-1/ICAM-1 or VLA-4/VCAM-1 interactions, and lectin adhesion molecule-1 and endothelial leukocyte adhesion molecule-1 appeared not to be implicated, other as yet undefined cell surface structures are likely to participate in T cell/EC interactions.

Lymphocyte traffic allows continuous immune surveillance of the body. In secondary lymphoid organs including lymph nodes, tonsils, adenoids, Peyer’s patches, appendix, and mucosa-associated lymphoid tissues leukocyte extravasation occurs predominantly in specialized postcapillary venules which are lined by high endothelium (see Reference 1 for review).

Some of the adhesive properties of high endothelium in inflamed tissue can be mimicked by exposing cultured human umbilical vein EC to cytokines such as TNF-α (2, 3), IL-1 (2, 3), or LPS (2). In man, leukocyte/EC interactions may therefore be studied with cytokine-treated allogeneic cultured umbilical vein EC.

Several sets of adhesion molecules, e.g., the ligand/receptor pairs leukocyte function-associated antigen (LFA)-1 intercellular adhesion molecule (ICAM)-1 (4), LFA-1/ICAM-1 (4), very late antigen (VLA)-4/vascular cell adhesion molecule (VCAM)-1 (6, 7), the lymphocyte homing receptor leucin adhesion molecule (LECAM)-1 (8, 9), endothelial leukocyte adhesion molecule (ELAM)-1 (10, 11), and the endothelial granule membrane protein-140 molecule (12) are involved in the antigen-independent interaction of EC and leukocytes. The human counterstructures of the last three molecules are as yet partly characterized (13). LFA-1 integrin expression on T cells from normal individuals strongly contributes to binding of these cells to their counterstructures on EC (11, 14, 15)

Accepted for publication November 22, 1991.

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5 Abbreviations used in this paper: EC, endothelial cell; PBS-BSA, PBS containing 0.01% CaCl2, 0.01% MgCl2 and 0.25% BSA; LFA, leukocyte function-associated antigen; ICAM, intercellular adhesion molecule; VCAM-1, vascular cell adhesion molecule-1; ELAM-1, endothelial leukocyte adhesion molecule-1; VLA, very late antigen; LAD, leukocyte adhesion deficiency; HEV, high endothelial venule; ECGF, endothelial cell growth factor.
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Monoclonal antibodies. The mAb used in the adhesion test and their final concentrations are listed in Table I. No differences were observed with the purified antibodies with the antibodies in addition to the mAbs used for phenotyping T cell clones and EC were obtained from several laboratories: CLB-10G11 (anti-CD3, 24) and CLB-14B12 (anti-CD8, 24) were purchased from Medarex Inc., West Lebanon, NH. Leu8 (anti-CD16) was obtained from Camera Biotech International, Buckinghamshire, UK; 1:2 mCi/×10^6 cells, followed by washing three times with DMEM) were added. Adhesion was allowed for 30 min at 37°C in the CO2 incubator. Non-adherent cells were removed by washing three times with 0.3 ml of PBS-BSA.

Inhibition experiments. EC and lymphocytes were preincubated with mAb and used without further washing.

Fluorescence analysis. A suspension of EC cultures was obtained by digestion of EC cultures with collagenase (125 U/ml in 137 mM NaCl, 4 mM KCl, and 10 mM HEPES, pH 7.0) for 30 min at 37°C. EC and T cells were washed in ice-cold PBS containing 0.25% NaN3 and suspended to 2×10^6 cells/ml. Fifty μl were transferred to 96-microwells and incubated for 30 min at 4°C with 50 μl 1/50 diluted mAb containing ascites. The analysis was performed in a FACScan (Becton and Dickinson, Mountain View, CA). Antigen expression was presented as the mean fluorescence intensity (arbitrary units).

RESULTS

Cell Surface Expression of T Cell Clones and EC

T cells. Analysis of cell surface molecules expressed by the cloned T cells J5132, J316, LAD1, LAD4, LAD6.6, and LAD19 is presented in Table II. No differences were observed between two cytotoxic LAD clones (LAD1 and LAD6.6) (P. Van de Wiel-van Kemenade, unpublished observations) and two non-cytotoxic LAD clones (LAD4 and LAD19). Furthermore, IL-2 and T cell–EC interaction plays a major role in the binding of T cells from LAD patients to EC activated for 24 h by TNF-α.

MATERIALS AND METHODS

EC. Human EC were isolated from umbilical vein by collagenase digestion (18). The cells were cultured in 2.0% gelatin-coated 75-cm² flasks (3275, Costar, Cambridge, MA) in M199 (Flow Laboratories, Irvine, Scotland) in the presence of penicillin and streptomycin (GIBCO Europe, Paisley, Scotland), 10% heat-inactivated human serum (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands), bovine pituitary extract (EGCG 5:100), boehringer Mannheim, FRG), heparin (10 μg/ml, Sigma, St. Louis, MO), BSA (0.25%, fraction V, boehringer Mannheim), and the ingredients transferrin (0.03%, bovine pancreas insulin (5 μg/ml), lipoic acid (1 μg/ml), oleic acid (1 μg/ml), and palmitic acid (1 μg/ml), all from Sigma), which have been described as components of a chemically defined medium for generating T cell clones (19). The cells were serially passed by trypsin treatment. Cells from passages 4 through 7 were used for the experiments. More than 95% of the cells were considered EC as they stained with mAb CLB-10G11 (anti-VLA-2, CDw49b) (44) and C17 (anti-β1-integrin, CD29) (50) from T. Springer, Boston; and B-G10 (anti-β3-integrin, CD41) (45] from A. Sonnenberg, Boston. Leu8 (anti-CD16) was obtained from Becton and Dickinson, San Jose, CA; 32.2 (anti-Fc receptor I, CD64) (59) and IV.3 (anti-Fc receptor II, CD32) were purchased from Medarex Inc., West Lebanon, NH. NK1.1 (anti-CD5, CD1b) (27) and HFI/3 (anti-VLA-4, CD49d) (37) have been described previously.

Adhesion test. Endothelial cells were trypsin treated, and 2×10⁴ cells in 100 μl of M199 containing 20% heat-inactivated human serum (but without ECGF, heparin, and the components of chemically defined medium; see above) were seeded into 0.2% gelatin-coated 96-well plates (3596, Costar). After incubation overnight the cells were stimulated with human rTNF-α (supernatant of cDNA-transfected COS cells) using the concentrations indicated in the experiments. Before initiation of the adhesion experiment the wells were washed twice with 0.3 ml of sterile PBS-BSA, and the wells were filled with 100 μl of DMEM containing 25 mm HEPES, pH 7.0, and 0.25% BSA. Subsequently, 50 μl containing 5,000 to 10,000 T cells that had been labeled with [51Cr]Na2Cr04 (350 to 600 mCi/mg; Nuclear Chicago, IL) were added to the EC. After 30 min at 37°C in the CO2 incubator. Non-adherent cells were removed by washing three times with 0.3 ml of PBS-BSA.

The adherent cells were solubilized for 30 min with 50 μl of 0.25% SDS, 1% Triton X-100, 0.5% sodium deoxycholate, and 5 mM EDTA and counted in a gamma counter (Multi-Flats 1, Packard Instrument Co., Downers Grove, IL). Inhibition was calculated according to the formula

% Inhibition = \frac{1 - \frac{cpm with mAb to test}{cpm with irrelevant control mAb}}{100}

For inhibition experiments EC and lymphocytes were preincubated with mAb and used without further washing.

Fluorescence analysis. A suspension of EC cultures was obtained by digestion of EC cultures with collagenase (125 U/ml in 137 mM NaCl, 4 mM KCl, and 10 mM HEPES, pH 7.0) for 30 min at 37°C. EC and T cells were washed in ice-cold PBS containing 0.25% BSA and 0.02% NaN3 and suspended to 2×10⁶ EC or 10×10⁶ T lymphocytes) cells/ml. Fifty μl were transferred to 96-microwells and incubated for 30 min at 4°C with 50 μl 1/50 diluted mAb containing ascites. The cells were washed with 2 ml of PBS containing 0.25% BSA and 0.02% NaN3 and incubated with 50 μl of affinity-purified goat anti-mouse IgG (Fab) (3rd generation) coupled to 100 μg/ml antibody; Zymed, San Francisco, CA). The cells were washed once in PBS containing 0.02% NaN3 in the presence of 0.25% BSA and once in PBS containing 0.02% NaN3 in the absence of BSA, fixed in 1.5% paraformaldehyde in PBS, and measured in the FACSscan (Becton and Dickinson, Mountain View, CA). Antigen expression was presented as the mean fluorescence intensity (arbitrary units).
not express the β2-chain of the leukocyte integrins (CD18). A striking feature was that in contrast to granulocytes and T lymphocytes that had been isolated from peripheral blood by centrifugal elutriation (not shown), the T cell clones lacked expression of LECAM-1, not only 1 wk after restimulation of the cells with allogeneic lymphocytes (feeder cells) and PHA but also after culturing the cells for another wk in the presence of IL-2 only.

EC. The expression of cell surface molecules on EC is shown in Figure 1. After stimulation of EC with rTNF-α VCAM-1 was expressed de novo whereas the expression of ICAM-1 (CD54) had increased markedly, and the expression of the Hermes-1 Ag (CD44) and HL-A-1 class I molecules had increased slightly. No expression was noticed of the HL-A class II molecules HL-A-DR/DQ or HL-A-DQ. Expression of the rapidly inducible adhesion molecule ELAM-1, which was highly expressed after 4 h of stimulation with rTNF-α (not shown), had faded away after 24 h in the presence of the cytokine.

**Comparison of adhesion of T cell clones to EC**

*Adhesion of LFA-1+/LFA-1+ T cells.* We first investigated the ability of different T cell clones to adhere to both unstimulated and rTNF-α-stimulated EC. Maximal adhesion of both LFA-1+ and LFA-1+ T cells was obtained after stimulation of EC with rTNF-α for 24 h (Fig. 2).
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Relative fluorescence

Figure 1. Phenotypic expression of surface molecules on EC as analyzed in the FACScan. EC were stimulated with 100 U/ml rTNF-α for 24 h (-----; unstimulated EC, -------).

Figure 2. Adhesion of T cell clones to EC. EC were cultured in the presence of rTNF-α [•, 100 U/ml; A, 200 U/ml] or medium (O, control). JS136 and HY827 are antigen-specific CD4+ LFA-1+ T cell clones; LAD4 and LAD19 are CD4+ LFA-1” T cell clones. The SE bars represent three independent tests within a representative of two experiments. To enable proper comparison, the tests with JS136 and LAD4 were performed simultaneously, as were the tests with HY827 and LAD19.

Although the LFA-1” T cell clones bound to untreated EC in much fewer numbers than the LFA-1” cells, the LFA-1” and LFA-1+ T cells showed a similar enhancement of adhesion when the EC were pretreated with rTNF-α (Figs. 2 and 3). From these results we conclude that although LFA-1” T cells showed reduced adhesion to EC because of absence of LFA-1/ICAM-1 and LFA-1/ICAM-2 interactions, T cells from LAD patients possess additional molecules that are involved in adhesion to cytokine-stimulated EC.

Cytotoxic/noncytotoxic T cells. No marked differences in adhesion to EC were obtained between the LFA-1+/CD4+ cytotoxic T cell clone JS136 and the tetanus toxoid-specific T helper cell clone HY827 (Fig. 2) or between the LFA-1”/CD4+ cytotoxic T cell clone LAD6.6 compared with the noncytotoxic T cell clones LAD4 and LAD19 (Fig. 3).

CD4/CD8 T cells. Adhesion to EC of the LFA-1+/CD4+ T cell clones JS136 and HY827 was similar to that of the LFA-1+/CD8+ T cell clone JS132. Likewise, adhesion of the LFA-1”/CD4+ T cell clones LAD4, LAD6.6, and LAD19 was similar to that of the LFA-1”/CD8+ T cell clone LAD1 (Fig. 3).

Structures involved in adhesion of T cell clones to EC

To investigate which molecules are responsible for T cell adhesion, we incubated T cells as well as EC with mAb before the adhesion assay. Effects on adhesion to EC of the LFA-1+/CD8+ cytotoxic T cell clone JS132 and the LFA-1”/CD8+ cytotoxic T cell clone LAD1, which, because of considerably slower growth rates than the CD4+ clones, were not available in sufficient amounts, were tested with a limited set of mAb (HLA class I, HLA-DR/DQ, CD3, CD8, LFA-1, p150,95, the ω-chain of the leukocyte integrins, the Hermes-1 Ag (CD44), and the vitronectin receptor). The inhibition of JS132 by mAb was comparable to that obtained with JS136 and HY827. Likewise, the inhibition of adhesion of LAD1 by mAb was comparable with that of LAD4, LAD6.6, and LAD19 (not shown). mAb to VCAM-1 was tested with JS136, HY827, and LAD19 (see below). No difference was noticed whether preincubation of T cells with mAb was performed at 4 or 37°C or whether preincubation of T cells and EC was performed for 30 min vs 60 min (compare Fig. 4 with Figs. 6 and 7, and Fig. 5 with Fig. 6).

LFA-1/ICAM-1. Several groups have reported that the leukocyte integrins play an important role in adhesion to EC. Notably, LFA-1 (CD11a) is involved in adhesion of unstimulated and phorbol dibutyrate-stimulated peripheral T lymphocytes to unstimulated EC (14, 15, 53, 54). In our experiments adhesion of LFA-1” T cell clones to
The antibody. *p = 0.01 and °p = 0.005. mAb to gpl00, a melanosomal protein in a one-sided t-test compared with the incubation lacking mAb, in which

JE136 and HY827 in Figs. 6 through 7). Inhibition of the counterstructures of LFA-1 on endothelial cells, with cloned T cells show that LFA-1 is the main structure involved in adhesion of T cells to unstimulated but not to rTNF-α-stimulated EC (24 h) and confirm the finding of others (14, 15, 53, 54) that PMA primarily stimulates LFA-1-dependent adhesion.

Role of VLA-4/VCAM-1. A structure on mouse lymphocytes showing homology with the human VLA-4 molecule is involved in adhesion to Peyer’s patches and mucosal HEV (55). Several investigators have reported that in man adhesion of T lymphocytes and some T and B lymphoid cell lines to TNF-α- or IL-1-stimulated EC can be inhibited by antibodies both against VLA-4 and its counterstructure. LFA-1-dependent adhesion.

These results indicate that LFA-1+ cell line Jurkat, adhere to rTNF-α-stimulated, but not to unstimulated, EC via the receptor/ligand pair VLA-4/VCAM-1. Since the adhesion of Jurkat cells to EC resembled more that of LFA-1+ than that of LFA-1+ T cells, the LFA-1 molecules on Jurkat cells are possibly not, or not fully, functional.

CD44. The Hermes-1 Ag is mainly involved in binding of PBL to mucosal and synovial lymph node and to a lesser extent to peripheral lymph node HEV (59). Previously, a different mAb (NKI-P1) to this Ag was prepared which also inhibited adhesion of PBL to peripheral lymphocytes showing homology with the human VLA-4 molecule is involved in adhesion to Peyer’s patches and mucosal HEV (55). Several investigators have reported that in man adhesion of T lymphocytes and some T and B lymphoid cell lines to TNF-α- or IL-1-stimulated EC can be inhibited by antibodies both against VLA-4 and its counterstructure. LFA-1-dependent adhesion.

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Monoclonal antibody

- JS136
- HYB27
- Jurkat
- LAD6.6
- LAD4

% T cell adherence

- unstimulated EC
- TNF-α-stimulated EC

Figure 6. Inhibition of adhesion of Jurkat, JS136, HYB27, LAD4, and LAD6.6 to EC by mAb directed against VLA-4 (CDw49d), LFA-1 (CD11a), the β2-chain of the leukocyte integrins (CD18), or a mixture of these mAb. Stimulation of EC was for 24 h with 100 U/ml rTNF-α. Preincubation of T cells with mAb was for 30 min at 4°C. The SE bars represent three independent tests within the experiment. A representative of two experiments is shown. See the legend of Figure 4 for an explanation of the asterisks.

Monoclonal antibody

- JS136
- LAD19
- LAD6.6
- LAD4

% T cell adherence

- unstimulated EC
- TNF-α-stimulated EC

Figure 7. Inhibition of adhesion of JS136, LAD6.6, and LAD4 to EC by mAb directed against VLA-4 (CDw49d), LFA-1 (CD11a), the β2-chain of the leukocyte integrins (CD18), ELAM-1, or a mixture of these mAb. Stimulation of EC was for 24 h with 100 U/ml rTNF-α. Preincubation of T cells with mAb was for 30 min at 4°C, and preincubation of EC with mAb to ELAM-1 was for 30 min at 37°C. The SE bars represent three independent tests within the experiment. A representative of two experiments is shown. See the legend of Figure 4 for an explanation of the asterisks.

node HEV but at the same time stimulated homotypic aggregation of cells of the EBV-transformed cell line JY (36). In tests with cloned T cells, however, we never observed inhibition of adhesion to (TNF-α-stimulated) EC. Instead, in 6 out of 14 experiments we observed more than 50% increased adhesion of cloned T cells by this mAb to unstimulated EC (Figs. 4 and 5). The increased adhesion was observed with both cytotoxic and noncytotoxic LFA-1+ and LFA-1− CD4+ T cell clones and was not dependent on preincubation of the T cells with anti-CD44 at 37°C compared with 4°C. The enhanced adhesion to unstimulated EC was only partially reduced by mAb to CD18 (Fig. 5). There are several explanations for this phenomenon, which will be discussed below.
In accordance with this finding, no inhibition of adhesion by anti-ELAM-1 mAb (Fig. 7), as discussed above, might not be activated. Preliminary experiments with mAb NKI-L16 directed against the α-chain of LFA-1, confirm this observation (Y. van Kooyk, unpublished observations).

Stimulation of LFA-1+ T cells by PMA increased adhesion of these cells to unstimulated EC, and this effect was completely LFA-1 dependent. This is in concordance with previous observations (66) and those of others (65), suggesting that LFA-1-dependent adhesion requires activation of LFA-1. In contrast, we repeatedly found that PMA was not able to enhance adhesion of LFA-1+ T cells to TNF-α-stimulated EC despite a much higher ICAM-1 expression on the EC. Similar results have been obtained by Kavanaugh et al. (58) with LFA-1+ T cell clones. This is in contrast to the data obtained with LFA-1+ T lymphocytes from peripheral blood, which, when activated with phorbol ester, showed enhanced adhesion to IL-1-stimulated EC (14, 53, 54). This enhanced adhesion of phorbol ester-activated LFA-1+ T lymphocytes to IL-1-
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These results suggest that phorbol ester-enhanced adhesion of resting LFA-1+ lymphocytes to IL-1-activated EC is also LFA-1 dependent. However, our findings suggest that compared with resting T lymphocytes from peripheral blood, LFA-1+ T cell clones use a different pathway for adhesion to activated EC.

We cannot completely rule out that the VLA-4/VCAM-1 pathway does not play a role in adhesion of the LFA-1+ T cell clones to EC. The possibility exists that an as yet unknown receptor/ligand pair contributes that much to adhesion of cloned T cells to EC that VLA-4/VCAM-1 interaction is obscured. However, other explanations are also possible, such as signals generated after the interaction of LFA-1 with ICAM-1 and/or ICAM-2, which may stimulate other adhesion pathways. A role for CD11b (which is highly expressed on JS136 cells) in adhesion to activated EC can, however, be excluded since mAb to the β2-subunit of the leukocyte integrins did not inhibit the interaction.

More experiments are necessary to prove this.

In a number of experiments the adhesion of LFA-1+ and LFA-1- T cells to unstimulated and rTNF-α-stimulated EC was markedly influenced by mAb to the Hermes-1 pl50,95 glycoprotein family and its molecular basis.