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T LYMPHOCYTE ADHESION TO ENDOTHELIAL CELLS: MECHANISMS DEMONSTRATED BY ANTI-LFA-1 MONOCLONAL ANTIBODIES[1]

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Adhesion of lymphocytes to vascular endothelium is the first event in the passage of lymphocytes into a chronic inflammatory reaction. To investigate molecular mechanisms of T-EC adhesion, monoclonal antibodies (Mab) against T cell surface antigens have been tested for inhibition of binding. Baseline and phorbol ester-stimulated adhesion were strongly inhibited by either Mab 60.3 (reactive with the β-chain of the LFA-1, OKM1, and p150,95 molecules) or by Mab TS 1/22 (specific for the α-chain of LFA-1). Although the increased binding of phorbol ester-stimulated lymphocytes was inhibited by anti-LFA-1 antibody, there was no increased expression of LFA-1 on phorbol ester-stimulated T cells, as determined by FACS analysis. Maximal inhibition of unstimulated and phorbol ester-stimulated T-EC adhesion was seen at Mab concentrations of 1 μg/ml. In contrast, LPS- and IL 1-enhanced T-EC adhesion were only weakly inhibited by these antibodies. Mab 60.3 and TS 1/22 did not stain either unstimulated EC or LPS- or IL 1-stimulated EC, as measured by FACS analysis; moreover, preincubation of EC alone with these antibodies did not lead to inhibition of T-EC binding. Adhesion was not affected by Mab against the sheep erythrocyte receptor (LFA-2), a nonpolymorphic HLA class 1 framework antigen, or against LFA-3, the α-chain of OKM1, or the α-chain of p150,95. These results suggest that the mechanism of binding of lymphocytes to unstimulated endothelium differs from that to stimulated endothelium. LFA-1 appears to be an important adhesion-related molecule for binding to unstimulated endothelium. However, the increased lymphocyte adhesion to IL 1- or LPS-stimulated EC observed in these experiments appears to be relatively independent of LFA-1. The increased adhesion to stimulated EC could be due either to an increase in the avidity or the density of the EC receptor molecules ordinarily involved in unstimulated T-EC binding or to the formation of alternative receptors on the stimulated EC that are not present on unstimulated cells.

The adhesion of lymphocytes to endothelium is the first step in the emigration of these cells from the blood into the tissues. This interaction can be studied in an in vitro model that measures the binding of ^{51}Cr-labeled human T cells to human umbilical vein endothelial cell (EC)[4] monolayers. Recent reports have demonstrated that T-EC adhesion can be enhanced by stimulation of T cells with phorbol esters (1) or by preincubation of EC with bacterial lipopolysaccharide (LPS) (2), interleukin 1 (IL 1) (3), or interferon-γ (IFN-γ) (4). Phorbol ester stimulation of T cells and LPS or IL 1 stimulation of EC lead to additive effects on T-EC binding (1). It has been suggested, on the basis of these observations, that these or similar factors may act in vivo to increase the adhesion of both activated and resting T cells to endothelium and thus augment their movement into inflammatory lesions (1).

The lymphocyte function-associated molecule, LFA-1, was first recognized by monoclonal antibodies (Mab) generated against cytotoxic T cell clones (5, 6). Initially, it was observed that Mab against LFA-1 inhibited the killing of target cells by cytolytic T cells (6), presumably by interfering with the binding of the T cells to their targets (7). It has since become evident that the involvement of LFA-1 in cellular interactions is not restricted to cytolytic T cells. Thus, Mab against LFA-1 have been shown to inhibit the spontaneous aggregation of Epstein Barr virus-transformed B lymphoblastoid cell lines (8) and phorbol ester-stimulated aggregation of peripheral blood mononuclear cells (9, 10) and lymphoid and myeloid cell lines (10). In addition, Mab against LFA-1 have recently been shown to inhibit the adhesion of interleukin 2-maintained T cells to EC (11).

To further define the molecular basis for T cell adhesion to EC, we have tested Mab against LFA-1 and other T cell surface antigens for their ability to inhibit the unstimulated and stimulated binding of peripheral blood T cells to EC. In this paper, we show that both the baseline binding and the binding of phorbol ester-stim-

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[4] Abbreviations used in this paper: EC, endothelial cell; LFA, lymphocyte function-associated antigen; HS, human serum; DPBS, Dulbecco's phosphate-buffered saline; $P(Bu)_2$, 4-β-phorbol-12-13-dibutyrate; DMSO, dimethyl sulfoxide; Mab, monoclonal antibody.

ulated T cells are largely inhibited by Mab against LFA-1. These Mab, however, were only weakly inhibitory for the binding attributable to stimulation of EC with LPS or IL 1, suggesting differences in the mechanisms of lymphocyte adhesion to unstimulated and stimulated endothelium.

MATERIALS AND METHODS

Preparation of umbilical vein EC. EC were obtained from human umbilical cords by collagenase (Worthington, Freehold, NJ) digestion by using a modification of a method by Jaffe et al. (12) as described (13). Cells from different cords were cultured separately in EC growth medium consisting of RPMI 1640 (Inland Laboratories, Austin, TX) supplemented with 15% fetal calf serum (FCS) (Gibco Laboratories, Grand Island, NY), 10% pooled human type O serum (HS) from normal blood donors, antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B; Inland Laboratories), 5 U/ml sodium heparin (Upjohn Co., Kalamazoo, MI), and 15 μg/ml EC growth factor (Collaborative Research, Lexington, MA) in tissue culture flasks pretreated with 1% gelatin. At confluence, cells were detached by using 0.125% trypsin (Gibco) and 1 mM EDTA. Cells in the monolayer were confirmed to be EC by immunofluorescent staining with a rabbit antifactor VIII antiserum (Cappel Laboratories, West Chester, PA). EC were used at the third or fourth passage. Less than 1% of the cells at these passages were stained by a monoclonal antimonocyte antibody (63D3) (14) as determined on a fluorescence-activated cell sorter (FACS).

Preparation of mononuclear cells. Peripheral blood was collected from normal donors by venipuncture and anticoagulated with heparin. The blood was diluted with an equal volume of Dulbecco's phosphate-buffered saline (DPBS) (Inland Laboratories) and underlaid with Ficoll-Hypaque (Histopaque-1077; Sigma Chemical Co., St. Louis, MO). After centrifugation for 30 min at 400 × G, the mononuclear cells were collected from the interface and washed three times in DPBS. Mononuclear cells were incubated in 15% FCS in RPMI with 10 mM HEPES (assay medium) in glass petri dishes (30×10^6 cells in 15 ml/dish) for 1 hr at 37°C in 5% CO_2. The nonadherent cells were then collected by gentle washing with assay medium. T lymphocytes were obtained from nonadherent cells, either by passage over nylon wool columns or by rosetting with neuraminidase-treated sheep erythrocytes and centrifugation over Ficoll-Hypaque. T cells purified by these two methods did not differ in their ability to bind to EC.

T cell preparations contained >94% 9.6 (15) staining cells (sheep erythrocyte receptor-positive lymphocytes), <1% 63D3 staining cells (monocytes/macrophages), 15 to 25% 60.1 (16) or OKM1 (17) staining lymphocytes, <10% S-HCL 3 (18, 19) staining cells (p150,95-positive lymphocytes), and <1% S-HCL 1 (20) staining cells (B cells), as determined by FACS.

^{51}Cr labeling. T cells from 60 to 120 ml of blood were suspended in 0.3 ml of assay medium plus 0.3 ml DPBS containing about 300 μCi Na_2CrO_4 (ICN Radiochemicals Co, Irvine, CA). After a 90-min incubation at 37°C with intermittent agitation, soluble ^{51}Cr and dead cells were removed by centrifuging five times through 10 ml of DPBS containing 5% (w/v) bovine serum albumin (BSA) (Fraction V: reagent grade; Miles Scientific, Naperville, IL). Cell viability was greater than 95% by trypan blue exclusion.

T cell to EC monolayer adhesion assay. EC at confluence were removed from culture flasks with 0.125% trypsin and 1 mM EDTA, centrifuged and resuspended in 15% FCS, 10% HS in RPMI 1640 at a concentration of 2×10^5 cells/ml. Samples (0.2 ml) were then cultured overnight or longer in flat-bottomed, gelatin-coated 96-well microtiter plates (Corning Glass Works, Corning, NY) until confluence was reached. After washing the EC, 2×10^5 ^{51}Cr-labeled lymphocytes in 0.2 ml assay medium were added per well. After incubation for 60 min at 37°C in 5% CO_2, the microwells were washed four times with 0.2 ml of warm assay medium to remove nonadherent lymphocytes. Next, 0.2 ml of an aqueous 1% solution of Triton X-100 (Sigma Chemical Co., St. Louis, MO) was added to each well, and the plate was reincubated for at least 10 min to lyse the remaining lymphocytes. The percentage of bound lymphocytes was then calculated as follows:

$$\% \text{ Lymphocytes bound} = \frac{\text{cpm in 0.1 ml lysate}}{\text{cpm in 0.1 ml of original lymphocyte suspension}} \times 100$$

The percentage inhibition of T-EC binding was calculated as follows:

$$\% \text{ Inhibition} = 1 - \frac{\% \text{ T-EC binding with Mab}}{\% \text{ T-EC binding in control}} \times 100$$

The percentage inhibition of T-EC binding enhancement attributable to stimulating agents was calculated as follows:

$$\% \text{ Inhibition} = 1 - \frac{\Delta \text{ in presence of Mab}}{\Delta \text{ in control without Mab}} \times 100$$

Where Δ = % T-EC binding in cultures with stimulating agent − % T-EC binding in control cultures without stimulating agent.

Reagents. Ultrapure human IL 1 was purchased from Genzyme, Boston, MA. 4-β-Phorbol-12-13-dibutyrate ($P(Bu)_2$) (Sigma Chemical Co., St. Louis, MO) was dissolved in dimethyl sulfoxide (DMSO) (1 mg/ml) and stored in the dark at −20°C. LPS (*E. coli* serotypes 0127:B8 and 0111:B4) was purchased from Sigma Chemical Co.

Monoclonal antibodies. Mab 60.3 (21), W6/32 (22), TS 1/22 (5, 6), TS 2/18 (5, 6), TS 2/9 (5, 6), 9.6 (15), OKM1 (17), and S-HCL 3 (18, 19) were purified from mouse ascites fluid by protein A affinity chromatography (23). Mab 60.1 (16) was purified from mouse ascites fluid by ammonium sulfate precipitation (24). Mab 63D3 (14) was a gift from Dr. Peter Stastny and was used as ascites fluid. S-HCL 1 (Leu-14) (20) was purchased from Becton Dickinson, Mountain View, CA.

Indirect immunofluorescence. Indirect immunofluorescence analysis of T lymphocytes and EC was performed by using Mab and fluorescein-conjugated $F(ab')_2$ fragments of goat anti-mouse immunoglobulin (Cappel Laboratories). Stained cells were fixed in 2% paraformaldehyde and analyzed by FACS within 7 days.

Statistical analysis. Differences between the result of experimental treatments were evaluated by means of the two-tailed Student's *t* test.

RESULTS

Inhibitory effect of Mab 60.3 on unstimulated and phorbol ester-stimulated T cell binding. The Mab 60.3 recognizes a cell surface glycoprotein complex that has been shown to be important in adhesion processes of neutrophils (25, 26), monocytes (27), and lymphocytes (9). When T cells were preincubated with Mab 60.3 and then incubated with EC in the continued presence of antibody, a dose-related inhibition of the binding of these unstimulated lymphocytes to unstimulated EC was observed (Fig. 1). Maximal inhibition was observed at concentrations of 1 μg/ml and 50% inhibition at 0.1 μg/ml. There was variation between experiments in the amount of inhibition observed with optimal concentrations of antibody, and complete inhibition was never seen. The mean inhibition in 31 separate experiments was 64.5%

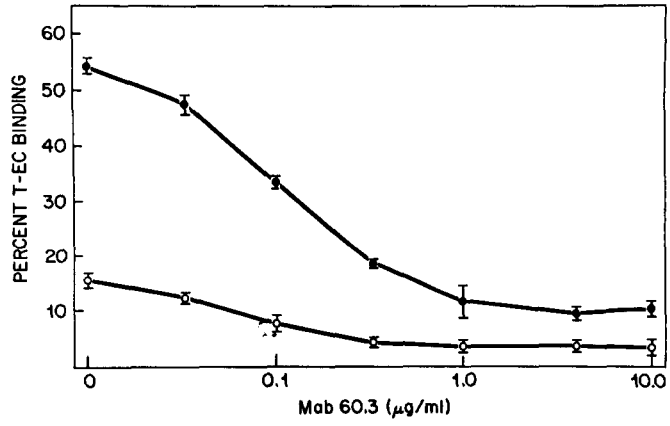

Figure 1. Inhibition of baseline and phorbol ester-stimulated T-EC binding by Mab 60.3. ^{51}Cr-labeled T cells were incubated at room temperature with increasing concentrations of Mab 60.3 or assay medium for 15 min before transferring to EC monolayers in the continued presence of the antibody. After addition of T cells, $P(Bu)_2$ (22 μl of 500 ng/ml, final concentration 50 ng/ml) (●—●) or an equivalent dilution of DMSO (0.05%) in assay medium (○—○) was added to the EC monolayers. Values = mean ± SD of triplicates.

(range 32.9 to 86.0%) compared with control binding with no added antibody.

Comparison of the effect of preincubation of either T cells or EC with Mab 60.3 showed that the action of the antibody was exerted on the T cells rather than the EC (Fig. 2). Moreover, 60.3 was shown by FACS analysis to bind to T cells, as was previously observed (21), and in agreement with findings in other studies (26, 27), not to bind to EC (data not shown). It was therefore concluded that the inhibitory effect of 60.3 on T-EC binding was due entirely to the masking of an adhesion-related molecule on the T cells.

Phorbol esters have been shown to stimulate T-EC adhesion by an action on the T cell (1). Phase-contrast microscopy demonstrated this enhanced adhesion to be due to an increased number of lymphocytes binding to EC rather than to an indirect effect of lymphocyte-lymphocyte aggregation. In 15 separate experiments, Mab 60.3 strongly inhibited the increase in T-EC binding due to phorbol ester stimulation of T cells by a mean of 75.7% (range of 53.9 to 93.5%). The dose-response relationship of the inhibition of phorbol ester-stimulated T-EC binding was similar to that of the inhibition of the binding of unstimulated T cells (Fig. 1), suggesting that phorbol esters increase T-EC adhesion by augmentation of a predominant mechanism involved in the binding of unstimulated T cells.

The effects of Mab to other T cell and EC surface components were compared with those of Mab 60.3. No inhibition of binding of unstimulated or phorbol ester-stimulated T cells was seen with the Mab W6/32 (40 μg/ml), which recognizes an HLA class 1 framework antigen (22) and, like 60.3, is an IgG2a immunoglobulin (Table I). It is of note that in spite of its failure to inhibit binding, the mean intensity of fluorescence of T cells treated with Mab W6/32 (at 40 μg/ml) was over five times as great as that of Mab 60.3 at 1 μg/ml on FACS analysis. Previous studies have shown that the conjugation of cytotoxic T cells with targets can be inhibited by Mab against the sheep erythrocyte receptor (LFA-2) or against LFA-3, a surface antigen present on T cells and EC (6). No inhibition of baseline or phorbol ester-stimulated binding was observed with the Mab TS 2/18 and 9.6, which react with LFA-2, or with the Mab TS 2/9, which reacts with LFA-3 (Table I).

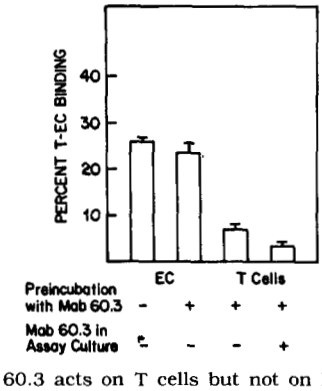

Figure 2. Mab 60.3 acts on T cells but not on EC to inhibit T-EC binding. ^{51}Cr-labeled T cells and EC were preincubated at room temperature with 60.3 (40 μg/ml) or medium control for 15 min. The T cells were then washed three times and resuspended in medium containing Mab 60.3 (40 μg/ml) or in assay medium alone as a control. The EC were in turn washed three times before addition of the T cells. Values = mean ± SD. Preincubation of only EC with 60.3 had no significant effect on T-EC binding. Preincubation of T cells with 60.3, but not including 60.3 in the assay, leads to significant ($p < 0.005$) inhibition of T-EC binding compared with both the non-antibody control and the culture in which EC were preincubated with 60.3. Preincubation of T cells with 60.3, followed by inclusion of 60.3 in the assay, was significantly ($p < 0.01$) more inhibitory than preincubation of T cells alone.

TABLE I

Inhibition of unstimulated and phorbol ester-stimulated T-EC binding by Mab 60.3[a]

Mab (μg/ml)	Percent T-EC Binding[b]	
	Unstimulated	Phorbol ester stimulated
No antibody	12.8 ± 1.8	36.9 ± 6.3
W6/32 (40)	14.9 ± 1.5	41.5 ± 4.2
9.6 (40)	13.8 ± 0.7	37.7 ± 3.0
TS 2/18 (40)	12.1 ± 1.0	39.6 ± 5.5
TS 2/9 (40)	10.0 ± 0.7	34.5 ± 4.7
60.3 (1)	4.6 ± 0.1[c]	11.9 ± 0.3[c]

[a] Mab 9.6 and TS 2/18 react with the sheep erythrocyte receptor (LFA-2) present on all T cells but not on EC; Mab W6/32 reacts with an antigen on the HLA class 1 framework; Mab TS 2/9 reacts with LFA-3 on T cells and EC.

[b] The ^{51}Cr-labeled T cells were preincubated for 15 min with Mab before transferring, in the continuous presence of Mab, to EC monolayers. Twenty-two microliters of 500 ng/ml $P(Bu)_2$ or 0.05% DMSO (each 10× final concentration) were added to wells 20 min before washing off nonadherent T cells.

[c] $p < 0.005$ compared with no antibody control.

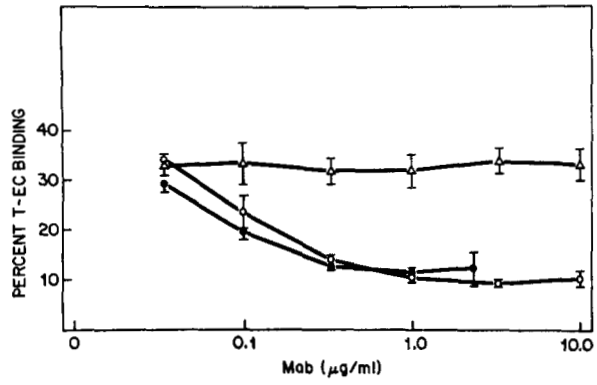

Figure 3. Comparison of inhibition of phorbol ester-stimulated T-EC binding by Mab 60.3 and TS 1/22. ^{51}Cr-labeled T cells were incubated at 37°C with $P(Bu)_2$ in assay medium for 20 min and then washed two times. The T cells were then incubated at room temperature with varying concentrations of Mab 60.3 (○—○), TS 1/22 (●—●), W6/32 (△—△), or assay medium alone for 15 min before transferring to EC monolayers in the continued presence of Mab. Values = mean ± SD. In the absence of Mab, $P(Bu)_2$ increased T-EC binding from a baseline of 12.7 ± 0.5% to 29.0 ± 0.7%. Saturating concentrations of 60.3 (10 μg/ml) and TS 1/22 (2.5 μg/ml) lowered the binding of unstimulated T cells to 4.7 ± 0.7% and 5.5 ± 0.8%, respectively. W6/32, at 40 μg/ml, had no inhibitory effect on the binding of unstimulated T cells.

Mab 60.3 and TS 1/22 inhibit T-EC binding by reacting with LFA-1. Mab 60.3 reacts with an epitope shared by LFA-1, the OKM1 antigen (also known as Mac-1 and Mo-1), and the p150,95 molecule. This epitope is thought to reside on the shared β-chain of these molecules (21, 28, 29). In contrast, Mab TS 1/22 reacts specifically with the α-chain of LFA-1 (αL). As shown in Figure 3, Mab 60.3 and TS 1/22 inhibited both unstimulated and phorbol ester-enhanced T-EC binding in a similar concentration-dependent manner. When 60.3 and TS 1/22 were used together, no significant additive effect on inhibition of binding of unstimulated or phorbol ester-stimulated T cells was observed (Table II). In contrast to the effects of Mab 60.3 and TS 1/22, Mab 60.1 and OKM1, which react specifically with the α-chain of the OKM1 antigen (αM), and Mab S-HCL 3, which reacts specifically with the α-chain of p150,95 (αX), had no effect on unstimulated or phorbol ester-enhanced adhesion (Table II). These obser-

TABLE II

60.3 and TS 1/22 Mab inhibit T-EC binding by reacting with the LFA-1; their effects are not additive[a]

Expt.	Mab (μg/ml)	% T-EC Binding[b]	
		Unstimulated	Phorbol ester stimulated
1	No antibody	12.8 ± 1.5	49.5 ± 3.7
	60.1 (40)	11.7 ± 1.5	44.4 ± 3.9
	60.3 (40)	6.5 ± 0.7[c]	15.5 ± 0.9[c]
	TS 1/22 (10)	6.4 ± 0.5[c]	16.5 ± 0.6[c]
2	No antibody	12.8 ± 1.8	36.9 ± 6.3
	60.1 (40)	13.4 ± 1.2	37.7 ± 4.9
	60.3 (1)	4.6 ± 0.1[c]	11.9 ± 0.3[c]
	TS 1/22 (1)	5.0 ± 0.5[c]	14.3 ± 1.3[c]
	60.3 (1) + TS 1/22 (1)	4.6 ± 0.2[c]	10.0 ± 1.1[c]
3	No antibody	15.9 ± 2.0	35.0 ± 3.0
	60.1 (20)	15.1 ± 1.3	36.7 ± 3.4
	S-HCL 3 (20)	14.6 ± 1.4	35.5 ± 2.8
	OKM1 (20)	15.2 ± 0.6	36.6 ± 3.4
	60.3 (2)	6.0 ± 0.4[c]	14.8 ± 1.8[c]
	TS 1/22 (2)	6.0 ± 1.4[c]	20.0 ± 1.3[c]
	60.3 (2) + TS 1/22 (2)	4.8 ± 1.7[c]	15.3 ± 1.2[c]

[a] Mab 60.1 and OKM1 react with αM on the OKM1 antigen. S-HCL 3 reacts with αX on the p150,95 antigen; TS 1/22 reacts with αL on LFA-1; 60.3 reacts with the common β-chain on OKM1, p150,95, and LFA-1.

[b] ^{51}Cr-labeled T cells were preincubated for 15 min with Mab before transferring, in the continuous presence of Mab, to EC monolayers. Twenty-two microliters of 500 ng/ml $P(Bu)_2$ or 0.05% DMSO (each 10× final concentration) were added to wells 20 min before washing off nonadherent T cells.

[c] $p < 0.005$ compared with no antibody control.

vations indicate that the inhibitory effect of 60.3 on T-EC adhesion is due to an effect on LFA-1 on the T cell surface, and is not due to masking of either the OKM1 antigen (30, 31) or the p150,95 molecule (18, 19) present on a subpopulation of the T11-positive cells.

Effect of Mab against LFA-1 on the T-EC binding enhanced by LPS or IL 1. In contrast to phorbol esters, LPS and IL 1 increase T-EC adhesion by actions on EC (1, 2). This enhanced adhesion was not due to stimulation of the expression of LFA-1 on EC, as determined by both FACS analysis and enzyme-linked immunosorbent assay (data not shown). When the effects of increasing concentrations of anti-LFA-1 Mab on the binding of T cells to unstimulated or LPS- or IL 1-stimulated EC were examined simultaneously, it was observed that the dose-inhibition curves of unstimulated and LPS- or IL 1-stimulated T-EC binding followed parallel courses. As shown in Figure 4, essentially only the baseline binding appeared to be inhibited, with no inhibition of the binding enhancement due to LPS. Thus, in 16 separate experiments of this type, Mab 60.3 inhibited the binding enhancement due to LPS by a mean of 14.8% (range −40.8 to 48.1%), and in 11 separate experiments, Mab 60.3 inhibited the binding enhancement due to IL 1 by a mean of 19.0% (range 8.2 to 45.0%). When Mab TS 1/22 and 60.3 were used in combination, no additional inhibition of LPS- or IL 1-enhanced T-EC binding was observed (data not shown). Thus, the inhibition by anti-LFA-1 Mab of LPS- and IL 1-enhanced binding was substantially less than the 64.5% mean inhibition of the binding of T cells to unstimulated EC or the 75.7% mean inhibition of the phorbol ester-stimulated increment in T cell binding. These observations suggest that a major part of LPS- and IL 1-enhanced binding may occur by a mechanism independent of LFA-1.

The effect of Mab 60.3 on combined phorbol ester stimulation of T cells and LPS treatment of EC was next

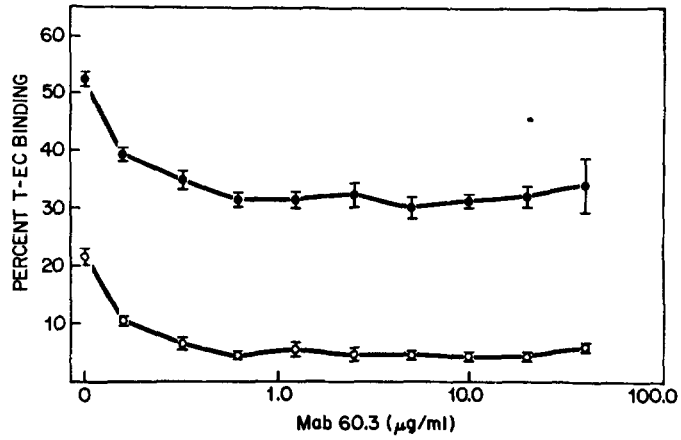

Figure 4. Effect of Mab 60.3 on LPS induced T-EC binding. ^{51}Cr-labeled T cells were incubated at room temperature with increasing concentrations of Mab 60.3 or assay medium alone for 15 min before transferring to EC monolayers in the continued presence of antibody. The EC had been preincubated with 500 ng/ml LPS (*E. coli* serotype 0111:B4) (●—●) or assay medium alone (○—○) for 4 hr and washed before addition of T cells. Values = mean ± SD. It is seen that although there was a parallel fall in binding of T cells to either stimulated or unstimulated EC on addition of Mab 60.3, the enhanced binding by LPS was not abrogated by the 60.3 Mab. (Similar results were obtained when EC monolayers were preincubated with IL 1, 0.5 U/ml for 4 hr.)

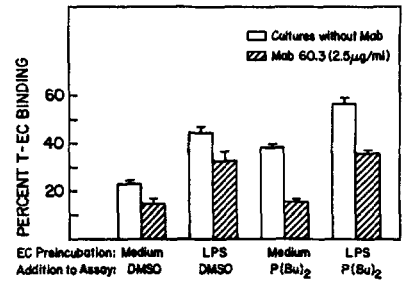

Figure 5. Comparison of effect of Mab 60.3 on phorbol ester and LPS enhanced T-EC binding. ^{51}Cr-labeled T cells were incubated at room temperature with 60.3 (at 2.5 μg/ml) or assay medium alone for 15 min before addition to the EC monolayers in the continued presence of Mab. EC were preincubated with 500 ng/ml LPS (*E. coli* serotype 0111:B4) or medium alone for 4 hr, and washed before addition of T cells. After addition of T cells, $P(Bu)_2$ (22 μl of 500 ng/ml, final concentration 50 ng/ml) or an equivalent dilution (0.05%) of DMSO in assay medium was added to the EC monolayers. Value = mean ± SD.

tested (Fig. 5). As might be expected from the fact that the effects of LPS and phorbol ester on T-EC binding were additive (1) and from the differing action of anti-LFA-1 Mab on LPS- and phorbol ester-enhanced binding, the amount of T cell binding to EC in the presence of Mab depended on the prior exposure of the EC to LPS and was not related to the presence or absence of phorbol ester in the culture. Thus, in the absence of LPS stimulation, T-EC binding values of 15% and 16% were found in unstimulated and phorbol ester-stimulated cultures, respectively; after LPS treatment, corresponding values were 33% and 35%, respectively. No inhibition of LPS- or IL 1-enhanced T-EC adhesion was seen with Mab TS 2/9, TS 2/18, 9.6, 60.1, OKM1, or S-HCL 3 (data not shown).

DISCUSSION

The epitope with which Mab 60.3 reacts is shared between the adhesion-related molecule LFA-1, present on virtually all peripheral blood leukocytes (6); the OKM1 antigen, present on monocytes and granulocytes and about 20% of T11-positive lymphocytes (30, 31); and the membrane glycoprotein p150,95, which is expressed on

monocytes and granulocytes and absent from the majority of lymphocytes (18). These molecules share a common β-chain of 95,000 m.w. but each has a distinct α-chain (29). It has therefore been concluded that Mab 60.3 is likely to react with the common β-chain (21, 28). However, a common epitope on the α-chains has not been definitively excluded.

We have shown in this report that the Mab 60.3 inhibits a mean of 64.5% (range 32.9 to 86.0%) of the binding of unstimulated T cells to unstimulated EC and a mean of 75.7% (range 53.9 to 93.5%) of the increase in T-EC binding due to phorbol ester stimulation of the T cells. Mab 60.3 has been shown in this and previous studies (26, 27) not to react with EC in culture. Moreover, preincubation of EC with 60.3 was not inhibitory for T cell binding. It can therefore be concluded that 60.3 inhibited T-EC binding by affecting T cells and not EC.

Mab 60.1 (16) and OKM1 (17, 30, 31), which react specifically with the α-chain of the OKM1 antigen (αM), and Mab S-HCL 3 (18, 19), which reacts specifically with the α-chain of p150,95, were not inhibitory for the binding of unstimulated or phorbol ester-stimulated T cells, suggesting that the action of Mab 60.3 was not directed at an OKM1-positive (30, 31) or p150,95-positive large granular lymphocyte cell subpopulation. Moreover, Mab TS 1/22 (5, 6), which reacts specifically with the α-chain of LFA-1 (αL) (29), was as effective as Mab 60.3 in inhibiting the adhesion of unstimulated and phorbol ester-enhanced T-EC binding, supporting the interpretation that Mab 60.3 inhibits T-EC binding through an action on LFA-1.

Mab against other lymphocyte cell surface antigens such as LFA-2 (the sheep erythrocyte receptor) (6, 15), LFA-3 (a surface antigen on T cells and EC) (5, 6), and the HLA class 1 framework were not inhibitory, even at saturating concentrations. Because at 40 μg/ml the mean fluorescent staining of W6/32 on the T cell surface, as observed by FACS analysis, was more than five times greater than that of 60.3 at its maximal inhibitory concentration of 1 μg/ml (data not shown), it seems unlikely that the inhibition of T-EC binding by Mab against LFA-1 was due to nonspecific modulation of the lymphocyte cell surface (32). Consistent with our interpretation that the inhibition of T-EC binding by Mab to LFA-1 reflects interference with a normal function of LFA-1 in T-EC adhesion is the reported inhibition of phorbol ester-induced lymphocyte aggregation by Fab fragments of 60.3 (9) or TS 1/22 (10), and the known existence of a heritable syndrome of LFA-1/Mac-1/p150,95 deficiency, in which leukocytes are deficient in a number of adhesion-related functions (25–27, 33, 34).

The observation that baseline and phorbol ester-enhanced T-EC binding are both inhibited by Mab against LFA-1 suggests that baseline binding is largely LFA-1 dependent and that phorbol esters increase T-EC binding by affecting either the number or the function of LFA-1 molecules on the T cell surface. Because it has not been possible to detect by FACS analysis any increased expression of LFA-1 on phorbol-treated lymphoblastoid cells (10), or, in the present study, on T cells after phorbol ester stimulation, it is probable that the effect of phorbol ester stimulation on lymphocyte LFA-1 is one of altered function. Phorbol esters have been shown to amplify the capping response of the lymphocyte surface membrane in response to contact with external ligands (35). As membrane capping can improve the affinity of surface receptors (36), phorbol esters might therefore increase T-EC adhesion by enhancing a capping response of lymphocyte surface adhesion molecules in response to contact with ligands on EC. It has not yet been determined whether LFA-1 is itself an adhesion molecule that reacts directly with EC or whether it is a surface molecule that is indirectly involved in phorbol ester-modulated adhesion (10).

Although T-EC binding was strongly inhibited by Mab against LFA-1, total inhibition of binding was not observed. In this respect, T cells appear to be similar to monocytes, in which spontaneous and phorbol ester-induced adhesion to EC are also incompletely inhibited by 60.3 (27).

In contrast to the inhibition of baseline and phorbol ester-stimulated binding, Mab 60.3 and TS 1/22 had little inhibitory effect on the T-EC binding enhancement by LPS and IL 1, which increase adhesion by acting on EC (2, 3). These results indicate that T cell adhesion to IL 1- or LPS-stimulated EC is substantially less dependent on LFA-1 than baseline and phorbol ester-enhanced binding. If LFA-1 is itself an adhesion ligand, the data would suggest the existence of an alternative ligand-receptor interaction by which T cells can adhere to IL 1- or LPS-stimulated EC. It remains possible, however, that IL 1 and LPS could change the density or avidity of the same receptors used for unstimulated binding in such a way that T cell adhesion is not blocked by Mab 60.3 and TS 1/22. Alternatively, if LFA-1 acts indirectly by modulating adhesion, the increased T cell binding to IL 1- or LPS-stimulated EC might similarly reflect an enhanced efficiency of adhesion on the part of the EC that is relatively independent of alterations in the lymphocyte membrane and that would not necessarily involve additional receptor-ligand interactions.

It has recently been demonstrated (37) that enhanced adhesion of neutrophils to IL 1- or LPS-stimulated EC is substantially inhibited by Mab 60.3. It may be, therefore, that different mechanisms govern the adhesion of neutrophils and lymphocytes to stimulated EC.

It is of interest that individuals whose leukocytes lack the LFA-1/OKM1/p150,95 glycoprotein family develop frequent infections in which inflammatory tissue is deficient in neutrophils (25, 33, 34). However, lymphocytes have been observed in such lesions (34), suggesting that lymphocyte migration into inflammatory tissue may not be wholly dependent on expression of LFA-1. It is possible that the adhesion system enhanced by IL 1 and LPS, which act on the EC (2, 3), could provide an alternative mechanism for the migration of LFA-1-deficient lymphocytes. IL 1-enhanced adhesiveness of EC may also explain why patients deficient in LFA-1 can still mount delayed cutaneous hypersensitivity reactions to skin test antigens (34).

In conclusion, we have shown that baseline and phorbol ester-stimulated adhesion of T cells to EC monolayers are inhibited by Mab against LFA-1, supporting the suggestion (11) that LFA-1 is an important adhesion-related molecule in determining the emigration of T cells from the blood into the tissue. IL 1- and LPS-induced adhesion, however, were only weakly inhibited by Mab against LFA-1, suggesting the existence of a second mechanism

that governs the adhesion of T cells to endothelium in inflammation. The LPS/IL 1-related mechanism may reflect a role of the local inflammatory tissue in stimulating lymphocyte migration into the tissue.

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