Involvement of Leukocyte Function-associated Antigen-1 (LFA-1) in the Invasion of Hepatocyte Cultures by Lymphoma and T-Cell Hybridoma Cells

Ed Roos and Folkert F. Roossien

Division of Cell Biology, Antoni van Leeuwenhoekhuis, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands

Abstract. We studied the interaction of MB6A lymphoma and TAM2D2 T cell hybridoma cells with hepatocyte cultures as an in vitro model for in vivo liver invasion by these tumor cells. A monoclonal antibody against leukocyte function-associated antigen-1 (LFA-1) inhibited adhesion of the tumor cells to the surface of hepatocytes and consequently strongly reduced invasion. This effect was specific since control antibodies, directed against Thy.1 and against T200, of the same isotype, similar affinity, and comparable binding to these cells, did not inhibit adhesion. This suggests that LFA-1 is involved in the formation of liver metastases by lymphoma cells.

TAM2D2 T cell hybridoma cells were agglutinated by anti-LFA-1, but not by control antibodies. Reduction of adhesion was not due to this agglutination since monovalent Fab fragments inhibited adhesion as well, inhibition was also seen under conditions where agglutination was minimal, and anti-LFA-1 similarly affected adhesion of MB6A lymphoma cells that were not agglutinated.

The two cell types differed in LFA-1 surface density. TAM2D2 cells exhibited 400,000 surface LFA-1 molecules, 10 times more than MB6A cells. Nevertheless, the level of adhesion and the extent of inhibition by the anti-LFA-1 antibody were only slightly larger for the TAM2D2 cells.

METASTATIC lymphoma cells often extensively colonize the liver (3, 8, 30, 38). Such lymphoma cells are highly invasive in cultures of isolated hepatocytes (32). Remarkably, normal activated T cells invade hepatocyte monolayers similarly as the tumor cells (29). When these T cells are fused with noninvasive and nonmetastatic lymphoma cells, the resulting T cell hybridomas are not only invasive but also acquire the capacity to metastasize, particularly to the liver (7, 31). This indicates that invasiveness is essential for metastasis formation by these cells, and suggests that one of the reasons for the high metastatic capacity of certain lymphoma cells is that they express the inherent invasive machinery of lymphoid cells. Furthermore, it implies that cell components involved in interactions between lymphocytes and tissues may play a role in invasion by lymphoma cells. Presently we report on the involvement of one such component, the leucocyte function-associated antigen-1 (LFA-1).[1]

LFA-1 was discovered with an antibody that inhibits killing by cytotoxic T cells (5). The antibody does not interfere with the lethal hit but rather with adhesion to the target cell, necessary for efficient killing (1, 6, 18, 42). It is now clear that LFA-1 is involved in multiple adhesion-related phenomena, mainly among leukocytes (6, 11, 14, 15, 23, 26, 33, 34, 42) but also in some cases between leukocytes and endothelial cells (4, 20, 47; for reviews, 17, 19, 43). The initial step of invasion into the hepatocyte monolayers is adhesion to the upper surface, and inhibition of this step leads to a corresponding reduction of invasion (22, 28). We now show that LFA-1 is involved in adhesion to hepatocytes by both a T cell hybridoma and a lymphoma cell line, and is therefore likely to be important for the formation of liver metastases by lymphoma cells.

1. *Abbreviation used in this paper*: LFA, leukocyte function-associated antigen.

Materials and Methods

Tumor Cells

MB6A lymphosarcoma cells were maintained in ascites in (C57Bl×DBA)F_1 mice as described (30). Metastasis formation in vivo and invasion in hepatocyte cultures in vitro by these cells have previously been described in detail (8, 30, 32). Generation, properties, and culture of mouse T cell hybridomas were reported elsewhere (31).

Hepatocyte Isolation and Culture

Rat hepatocytes were isolated as described previously (28). The cells were seeded in 16-mm multiwell plates (Primaria; Falcon Labware, Oxnard, CA) at 2.5 × 10^5 cells per well in DME (Flow Laboratories, Irvine, Ayeshire, Scotland) supplemented with 5 μg bovine insulin/ml (Sigma Chemical Co., St. Louis, MO). Before seeding, the wells were coated with 5% newborn

calf serum (Sera Laboratories, Sussex, UK) in PBS. After 2 h, the cells were washed twice with DME-insulin, and cultured overnight in the same medium supplemented with 10% newborn calf serum.

Adhesion and Invasion Assays

Adhesion of tumor cells to hepatocyte cultures was quantitated using tumor cells labeled with ^{51}Cr (100 μCi $Na^{51}CrO_4$, 1 mCi/μg Cr; Amersham International, Amersham, UK) per 2×10^7 cells in 100–200 μl PBS for 1 h at 37°C, followed by three washes in PBS. After overnight culture, hepatocytes were washed once with Earle's balanced salt solution (EBSS; Flow Laboratories). 2–4 $\times 10^5$ labeled tumor cells were incubated for 1 h at 37°C, then washed three times with PBS, and sucked dry. Subsequently, 400 μl 1 N NaOH was added. Radioactivity in the supernatant was counted when the hepatocytes were completely detached. To determine the possible effects of antibodies, the tumor cells were preincubated for 30 min in 400 μl of a particular dilution of antibody in EBSS at 37°C and subsequently this 400 μl was transferred to a hepatocyte culture well. Control cells were similarly incubated with EBSS only. In some experiments the effect of preincubation was compared with that in the continued presence of antibody. Cells were preincubated as above, centrifuged, and the supernatants removed. The pellets were washed with PBS, and either EBSS or the original supernatant containing antibody was added, and the suspensions were transferred to hepatocyte culture wells. All measurements were done with at least two control measurements per plate.

Adhesion, and in addition invasion, was also quantitated by light microscopy, as described previously (28). Briefly, 6–8 $\times 10^5$ tumor cells either incubated or not with antibody as above, in 400 μl EBSS, were added per hepatocyte culture in a 16-mm well. The cultures were incubated at 37°C for 4 h, washed once with PBS, fixed with glutaraldehyde, dehydrated, and scraped from the dish. The culture fragments were pelleted and embedded in Epon (Ladd Research Industries, Burlington, VT). Sections 1 μm thick were stained and observed with a light microscope. Hepatocyte nuclei, tumor cells adhering to the upper surface of hepatocytes, and infiltrated tumor cells were counted. The "interaction index" = (adherent + infiltrated tumor cells/hepatocyte nuclei) is a measure of total interaction, i.e., adhesion to the upper surface plus infiltration; the "infiltration index" = (infiltrated cells/hepatocyte nuclei) a measure of invasion. Measurements were performed in duplicate or triplicate, with at least two controls per plate.

Antibodies

The monoclonal antibody used was M17/4 (5), a rat IgG2a, directed against mouse LFA-1 α-chain. The two control antibodies were also rat IgG2a's: M1/9.3 (43), directed against mouse T200, and M5/49 (43), against mouse Thy.1. The antibodies were kindly provided by Dr. E. Martz (University of Massachusetts). Hybridoma supernatants were precipitated with 50% saturated $(NH_4)_2SO_4$, the pellets taken up in 0.9% NaCl + 10 mM Hepes pH 7.4, dialyzed against the same buffer, and stored at −20°C. Part of the experiments were done with dilutions of these preparations. Alternatively, antibodies were purified from supernatants or precipitates by affinity chromatography on Sepharose-coupled goat anti-rat Ig antibodies (Nordic Immunology, Tilburg, The Netherlands). The eluate (0.58% acetic acid in 150 mM NaCl) was immediately neutralized with 1 M Tris, concentrated in a filter unit (Amicon, Danvers, MA) (cut off: M = 10,000), and dialyzed against PBS. The concentration was measured spectrophotometrically at 280 nm using purified rat IgG (Nordic Immunology) as standard. These preparations contained pure antibody as determined by SDS-PAGE (not shown).

Preparation of Fab Fragments

Fab fragments were prepared essentially according to Rousseaux et al. (36). Antibodies were dialyzed against 75 mM NaCl and 2 mM EDTA in 75 mM sodium phosphate, pH 7.0 and concentrated to 1–2 mg/ml. Papain was added to 1% (wt/wt) of the amount of antibody, cystein to a final concentration of 0.5 M, and the mixture was incubated overnight at 37°C. Digestion was stopped by the addition of iodoacetamide to a final concentration of 50 mM. This preparation was dialyzed against PBS and stored at −20°C. Analysis by SDS-PAGE showed that all antibody had been digested (not shown).

Antibody Binding Assays

Binding of monoclonal antibodies to tumor cells was assessed indirectly using ^{125}I-labeled anti-rat Ig antibodies (Amersham International). To conical bottom microtiter plates (Greiner, Nürtingen, Germany) 2–4 $\times 10^5$ cells in 100 μl culture medium were added per well. The plates were centrifuged at 2,000 rpm for 2 min, the supernatant was replaced with 100 μl of monoclonal antibody diluted in PBS (37°C), and the pellets dispersed. The plates were incubated at 37°C for 30 min, centrifuged, washed with ice-cold PBS, the pellets dispersed in 100 μl ^{125}I-anti-rat Ig (16 ng/ml PBS, 15 μCi/μg), incubated on ice for 30 min, centrifuged, washed three times with cold PBS, and finally the pellets were taken up in PBS, transferred to counting vials, and radioactivity was counted. The radioactivity of the blanks (PBS instead of monoclonal antibody), usually less than 200 cpm, was subtracted from the values obtained. A normal saturation curve was obtained and was converted to a straight line by plotting 1/cpm vs. 1/concentration. In a few cases, binding was assessed by fluorescence-activated cell sorting analysis, essentially as described previously (31). The obtained saturation curves were converted to straight lines by plotting 1/relative fluorescence vs. 1/concentration.

Binding of anti-LFA-1 antibodies was also assessed directly. 25 μg of purified anti-LFA-1 antibody was reacted with 1 mCi $Na^{125}I$ using chloramine T, and separated from free iodide by chromatography on a Dowex column (Bio-Rad Laboratories, Richmond, CA) (12). The assay was performed in conical bottom microtiter plates as described above. After incubation at 37°C for 30 min with the labeled reagent, the plates were centrifuged, the supernatants transferred to counting vials, the wells washed once with PBS, and the wash fluid combined with the supernatants. The pellets were taken up in PBS, transferred to vials, and the radioactivity in both supernatants and pellets was counted. Binding in the presence of a 100-fold excess of unlabeled antibody was 2–3% of that in the absence of competition. The amount higher than 1% was considered nonspecific binding and was subtracted from the obtained values.

Assay of Antibody Concentration

The concentration of antibody in the ammonium sulphate precipitates was determined by a solid phase radioimmunoassay. Polyvinyl chloride microtiter plates (type M-24-A; Dynatech, Billingshurst, UK) were coated with goat anti-rat IgG (Nordic Immunology), 100 μg/ml PBS, 100 μl per well, for 30 min at 20°C, and subsequently with 300 μl 0.5% BSA (Sigma Chemical Co.) in PBS for 30 min at 20°C. The well contents were replaced with serial dilutions of purified anti-LFA-1 antibody and of antibody samples to be measured, and the plates incubated for 30 min at 20°C. After washing twice with PBS, the wells were exposed to 100 μl of ^{125}I-labeled sheep anti-rat IgG antibodies (16 ng/ml, 15 μCi/μg; Amersham International) for 30 min at 37°C. After washing twice with PBS, the wells were sucked dry, individual wells were cut from the plates, and radioactivity counted. Nonspecific binding, as measured in control wells exposed to PBS instead of the primary antibody, was subtracted. The saturation curve obtained for the purified antibody was converted to a straight line by plotting 1/cpm vs. 1/concentration. This plot was used to determine concentrations in the samples.

Immune Precipitation

Plasma membrane proteins were labeled by incubation of 3×10^6 cells

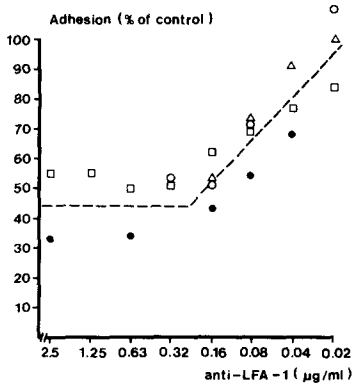

Figure 1. Effect of anti-LFA-1 antibodies on adhesion of ^{51}Cr-labeled MB6A lymphosarcoma cells to hepatocyte cultures. Values obtained in four different experiments are denoted by different symbols. The values given are percentages of adhesion of control cells on the same 24-well plate, and averages of triplicates. Adhesion was assessed 1 h after addition of the cells.

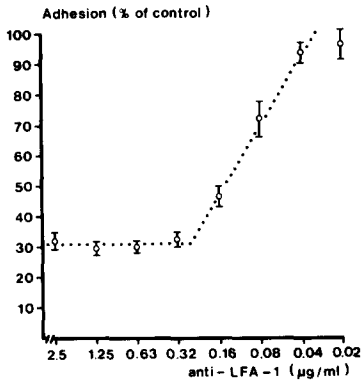

Figure 2. Effect of anti-LFA-1 on adhesion of TAM2D2 T cell hybridoma cells to hepatocytes in a representative experiment. Given are averages of triplicates and standard deviations. The procedure was slightly different for the T cell hybrids to minimize agglutination, as described in the text.

with 1 mCi $Na^{125}I$ (Amersham International) in pyrex tubes coated with Iodogen (Pierce Chemical Co., Rockford, IL). After 10 min the reaction was terminated by suspending the cells in an excess of cold PBS containing 20 mM KI. Cells were washed twice with cold PBS and stored as pellets at −70°C. Labeled cells (10^7) were lysed in 1 ml of 0.5% NP-40 in 40 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, and 0.002% soy bean trypsin inhibitor (all reagents from Sigma Chemical Co.). After 15 min on ice, cell debris was removed by 10-min centrifugation in a minifuge. Lysates were precleared by subsequent incubation with 10 μg rat IgG (Nordic Immunology) and 80 μg of Sepharose-coupled anti-rat IgG antibodies (Nordic Immunology) for 60 and 90 min, respectively. After overnight incubation of the lysate with anti-LFA-1 antibodies (30 μg/ml), the immune complexes were precipitated with 300 μg/ml of Sepharose-coupled goat anti-rat IgG antibodies for 2 h. The precipitates were washed six times with 0.5% NP-40 in 50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. SDS-PAGE was performed according to Laemmli (16). Precipitates were suspended in solubilization buffer, incubated for 5 min at 100°C, centrifuged to remove the Sepharose, and subjected to electrophoresis on 7.5% polyacrylamide gels.

Results

Effect of Antibodies on Adhesion of Tumor Cells to Hepatocytes

Monoclonal antibody M17/4 (5), directed against LFA-1, was found to inhibit adhesion of MB6A lymphosarcoma cells to rat hepatocyte cultures. As shown in Fig. 1, adhesion as measured with radioactively labeled cells was reduced to 40–60% of controls, with maximal inhibition down to 200 ng/ml and half-maximal inhibition at ~80 ng/ml. The results of four different experiments are depicted to show the extent of variability. We used either purified antibody or ammonium sulphate precipitates with similar results. Concentrations of antibody in the latter preparations were measured by a solid phase radioimmunoassay.

The level of inhibition was independent of the extent of adhesion in the control wells, which varied between 15 and 30% of added cells. As a control antibody we used M1/9.3 (43), also a rat IgG2a, directed against T200. This antigen is present on MB6A cells in similar amounts as LFA-1, and the affinity of the antibody is comparable to M17/4 (see below). This control antibody did not affect or even slightly stimulate adhesion (data not shown).

Agglutination of T Cell Hybridomas

A similar reduction of adhesion was seen with the invasive and metastatic T cell hybridoma TAM2D2 (31). However, quite in contrast to MB6A cells, the hybridoma cells agglutinated during the incubation period. This agglutination might conceivably have influenced adhesion by reducing the number of single hybridoma cells available for interaction with hepatocytes. To overcome this problem, the cells were preincubated at 20 rather than 37°C and also allowed to settle on the hepatocytes at 20°C for 15 min before incubation at 37°C. Under these conditions agglutination was absent or negligible during the following hour. Yet, a similar inhibition of adhesion was observed, down to 30% of control values (20–40% of added cells) with a concentration dependence comparable to that seen with MB6A cells (Fig. 2).

In addition to anti-T200, antibody M5/49 (43) was used as a control. Both antibodies did not influence adhesion. M5/49 is also a rat IgG2a and reacts with Thy.1, which is approximately equally abundant on TAM2D2 cells as T200 and LFA-1 (see below). Further evidence against agglutination being the cause of inhibition was the effect of Fab fragments (see below). Agglutination occurred with most but not all of a set of T cell hybridomas (31) tested, independent of adhesiveness and invasive potential.

Effect of Fab Fragments

The effect of monovalent M17/4 anti-LFA-1 Fab fragments on adhesion of MB6A and TAM2D2 cells in a representative experiment is shown in Fig. 3. Maximal inhibition, ~50%, was seen at 10 μg/ml, a much higher concentration than with whole antibody. Half-maximal inhibition occurred at different concentrations for the two cell types: 3.5 μg/ml for TAM2D2 and 1.5 μg/ml for MB6A cells. In some experiments cells were only preincubated and antibody or Fab fragments were not present in the incubation medium. As shown in Table I, preincubation only with the Fab fragments did not lead to inhibition. With whole anti-LFA-1 antibody the effect was reduced as compared with when the antibody had been continuously present.

Effect of Anti-LFA-1 on Invasion

The effect of antibodies on adhesion as well as on invasion was also determined by counting adherent and infiltrated cells in sections of embedded cultures. The observation period was usually extended to 4 h to allow for extensive invasion of the cells into the hepatocyte cultures. Both adhe-

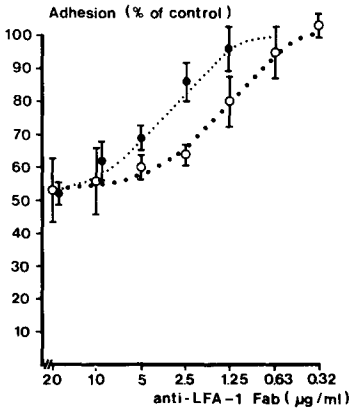

Figure 3. Effect on adhesion of anti-LFA-1 Fab fragments. (*Closed circles*) TAM2D2 cells; (*open circles*) MB6A cells.

Table I. Effect of Anti-LFA-1 in the Continued Presence of Antibody Compared with Preincubation Only

		Adhesion (% of control)	
	Concentration	Continuous presence	Preincubation only
	μg/ml		
Anti-LFA-1 antibody	0.16	36.7 ± 1.2	64.1 ± 1.4
	0.08	60.3 ± 3.3	87.1 ± 0.7
Anti-LFA-1 Fab	20	44.5 ± 2.9	102.4 ± 1.3
	10	55.8 ± 4.0	101.0 ± 2.5

^{51}Cr-labeled TAM2D2 cells were preincubated with anti-LFA-1 antibody or Fab fragments at the indicated concentrations. The cells were then added to hepatocyte cultures either in the preincubation medium or in fresh medium without antibodies (for details see Materials and Methods). The amount of label attached to the hepatocytes was compared with that in control cultures (cells not exposed to antibody). The data given are from one of two experiments with identical results.

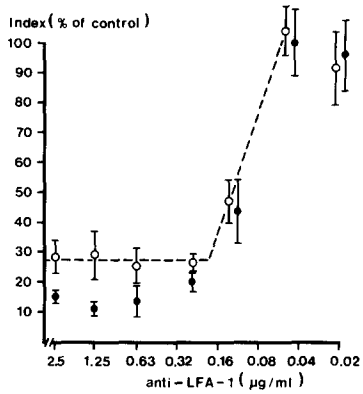

Figure 4. Inhibition of adhesion and invasion of TAM2D2 cells in hepatocyte cultures 4 h after addition of the cells as determined by counting cells in sections of embedded cultures (see Materials and Methods). (*Open circles*) The interaction index and (*closed circles*) the infiltration index (for explanation see Materials and Methods), as percentage of control values. Shown are the data for one of several experiments with similar results.

sion and invasion of MB6A as well as TAM2D2 cells were strongly inhibited. The effect was similar for both cell types although agglutination occurred with TAM2D2 and not with MB6A cells. The results of a representative experiment with TAM2D2 cells are shown in Fig. 4. Reduction of adhesion was larger than seen with radiolabeled cells, down to 20% of controls. This was not due to the difference in incubation period, because a similar reduction of the interaction index was also seen in cultures fixed after 1 h. It should be noted that this assay ignores all tumor cells present that do not really adhere to viable hepatocytes. Furthermore, the cultures are very extensively washed during dehydration, and the culture fragments centrifuged, possibly resulting in more extensive detachment of weakly adherent cells. As can be seen in Fig. 4, invasion was sometimes reduced to a larger extent than adhesion. However, in most experiments the percentages of inhibition were comparable. Reduction of invasion can therefore largely be attributed to the interference with adhesion to the upper surface of the hepatocytes, the initial step in the invasion process.

Binding of Antibodies and Fab Fragments to Tumor Cells

Scatchard plots of binding data obtained with ^{125}I-labeled anti-LFA-1 antibodies for MB6A and TAM2D2 cells are shown in Fig. 5. The dissociation constant calculated from the two plots was 0.93×10^{-9} M or 1.06×10^{-9} M, respectively, and the calculated numbers of LFA-1 molecules per cell were 4×10^4 for MB6A and 4×10^5 for TAM2D2. Binding was also assessed indirectly using ^{125}I-labeled anti-rat IgG as secondary antibody. This yielded a saturation curve, which was converted into a straight line by plotting 1/cpm vs. 1/concentration, as shown in Fig. 6 for TAM2D2 cells. The dissociation constant, as deduced from the intercept with the *x*-axis, was 1.03×10^{-9} M, essentially the same value as measured directly. The 10-fold difference in LFA-1 density was in reasonable agreement with the 18 times lower amount of LFA-1 immune precipitated from MB6A cells: 340 cpm as compared with 6,200 cpm per 10^6 cells iodinated under the same conditions (Fig. 7).

Binding of Fab fragments was only assessed by the indirect method (not shown), because for unknown reasons very high nonspecific binding occurred with ^{125}I-labeled Fab fragments. The dissociation constant was estimated to be 0.71×10^{-8} M. In this case binding was also measured by fluorescence on the fluorescence-activated cell sorter (not shown). This yielded a dissociation constant of 0.80×10^{-8} M.

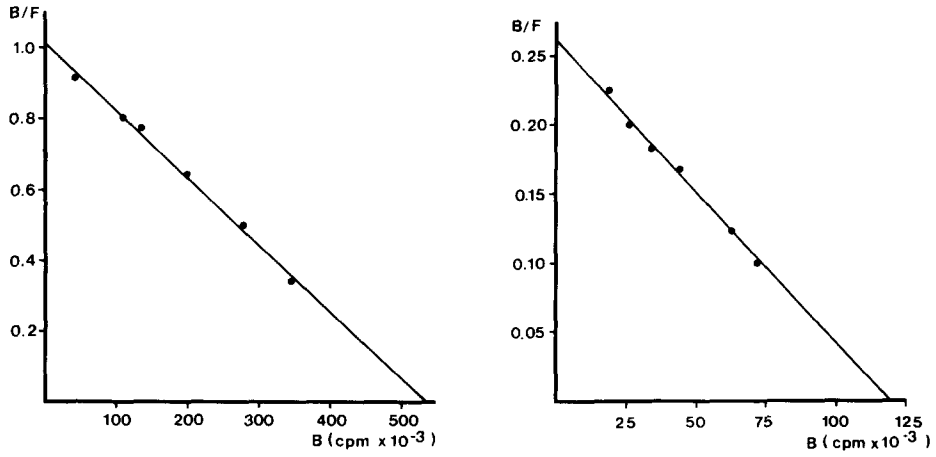

Figure 5. Scatchard plots obtained with ^{125}I-labeled anti-LFA-1 antibodies (specific activity: 3.3×10^{10} cpm/mg = 5×10^{12} cpm/μmol) for 4×10^5 MB6A cells (*left*) and 1.7×10^5 TAM2D2 cells (*right*).

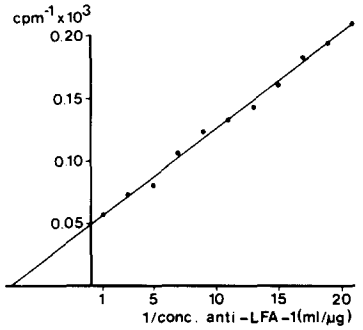

Figure 6. Binding of anti-LFA-1 antibody to 2 × 10^5 TAM2D2 cells, as determined by an indirect assay. Cells were exposed to various concentrations of antibody and then to ^{125}I-labeled anti-rat IgG. The saturation curve obtained was converted to a straight line by plotting 1/cpm vs. 1/concentration of antibody. Dissociation constants and binding levels were derived from such plots and compared for anti-LFA-1 and the control antibodies (Table II).

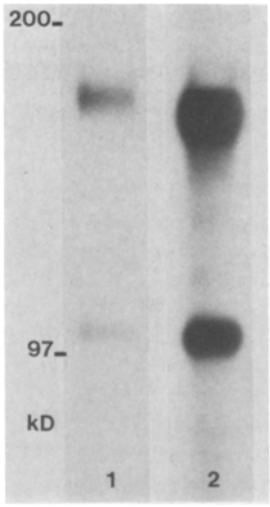

Figure 7. SDS-PAGE patterns of anti-LFA-1 immune precipitates from 3.3 × 10^6 MB6A (*left*) and TAM2D2 (*right*) cells.

Thus, the dissociation constant of Fab fragments was approximately eight times larger than that of whole antibody. Since the two control antibodies against T200 and Thy.1 were of the same isotype as the one against LFA-1, it was possible to compare binding to these three molecules by the indirect method. Table II gives the results. It is evident that both the extent of binding and the affinity of the control antibodies is at least comparable to anti-LFA-1.

Discussion

A monoclonal antibody against LFA-1 was found to inhibit adhesion of highly malignant MB6A lymphoma and T cell hybridoma cells to the upper surface of hepatocyte monolayers and thus to reduce invasion into those monolayers, whereas appropriate control antibodies had no effect. Since these highly invasive cells readily form metastases, in the liver and spleen for MB6A cells (8) and both in the liver and many other sites for the T cell hybrids (30), and since noninvasive hybrids do not metastasize, this finding indicates an involvement of LFA-1 in the formation of liver metastases.

LFA-1 is known to be involved in multiple cellular interactions, mainly between leukocytes (6, 11, 14, 15, 23, 26, 33, 34, 42), and in some cases also between leukocytes and endothelial cells (4, 20, 47). Cytotoxic T lymphocyte killing of fibroblastic or epithelial target cells was found to be LFA-1 independent (40). We were therefore surprised to find that LFA-1 is necessary for efficient adhesion to an epithelial cell. Apparently, the hepatocyte expresses an LFA-1 counterstructure (17) and in this respect may be unique among epithelial cells. If so, this might be one of the reasons for the predilection of many lymphoma cells for the liver (2). For instance, the MB6A cells used here metastasize only to spleen and liver (8). Recently a putative human LFA-1 counterstructure termed ICAM-1 (35) was identified. This molecule was shown not to be present on human hepatocytes (9). If human and rat hepatocytes are comparable in this respect, our results suggest that more than one LFA-1 counterstructure exists.

Our experiments were hampered by anti-LFA-1-induced agglutination of the T cell hybridoma cells, a phenomenon not described for other cell types (6). On the contrary, anti-LFA-1 has been reported to inhibit spontaneous (21, 26) and 12-*O*-tetradecanyl phorbol-13-acetate (TPA)-induced (34) aggregation of lymphoblastoid cell lines. Inhibition of adhesion was not the trivial result of agglutination because in 1-h tests the effect persisted under conditions where agglutination was minimal. In the 4-h tests performed to measure invasion, agglutination could not be avoided, but was probably not of influence because the effect was completely comparable for MB6A lymphoma cells which did not agglutinate. Furthermore, monovalent Fab fragments also inhibited but did not induce agglutination. The concentration required was much higher than that of whole antibody but this can be attributed to the measured eightfold lower affinity of the Fab fragments, as also demonstrated by the lack of inhibition if cells were only pretreated, so that the Fab fragments were not present during the interaction period (Table I).

It is remarkable that the two control antibodies directed

Table II. Affinity and Binding Levels of Antibodies

		Maximal binding		
	K_d	TAM2D2	MB6A	TAM2D2/MB6A
	M	*10^5 cpm/10^6 cells*	*10^5 cpm/10^6 cells*	*10^5 cpm/10^6 cells*
Anti-LFA-1 (M17/4)	1 × 10^{-9}	2.87	0.33	8.7
Anti-T200 (M1/9.3)	1 × 10^{-9}	2.76	0.42	6.4
Anti-Thy.1 (M5/49)	4 × 10^{-10}	2.59	–	–

Cells were exposed to the indicated antibodies at various concentrations, and subsequently ^{125}I-labeled anti-rat IgG, as detailed in Materials and Methods. From plots as in Fig. 6, maximal binding at saturating antibody concentrations and the half-maximal binding concentration were derived, and from the latter value the dissociation constant was calculated.

against T200 and Thy.1 did not agglutinate the hybrid cells although they were of the same isotype, had at least similar affinity, and exhibited similar binding levels. Apparently, the interaction of the anti-LFA-1 antibody with the cells is qualitatively different from the control antibodies. The reason for this remains unexplained. Recently, it was shown that binding of particular anti-LFA-1 antibodies can activate cells. On B-cells the effect was similar to that of B-cell stimulatory factor-1 (24), and in T-cells proliferation and interleukin release was induced (27), implying that LFA-1 is a receptor able to transduce signals into the cell. The induction of agglutination might conceivably be due to the generation of such a signal.

The extent of adhesion of TAM2D2 and MB6A cells to hepatocytes varied considerably between experiments but was roughly comparable for the two cell types (20–40 and 15–30% of added cells in 1 h, respectively). The extent of inhibition of adhesion by anti-LFA-1 antibody was also similar or only slightly larger for TAM2D2 cells and independent of the extent of adhesion in individual experiments. This similarity is remarkable in view of the 10-fold higher LFA-1 density on the TAM2D2 cells. Apparently, there is no relation between the amount of free surface LFA-1 and LFA-1-dependent adhesion. This was also found for a series of different T cell hybridomas (data not shown).

This lack of correlation has also been reported for other cell types. For instance, cytotoxic macrophages require LFA-1 for enhanced adhesion to tumor target cells, yet express as much LFA-1 as endotoxin-primed macrophages, which are not cytotoxic and do not exhibit enhanced adhesion (44, 45). The tumor promoter TPA induces this enhanced adhesion to target cells (41) as well as LFA-1-dependent aggregation of lymphoblastoid cells (34) while it does not alter LFA-1 expression. This suggests that LFA-1 function depends on the activity of signal transmission components like protein kinase C, the TPA receptor (25). LFA-1 belongs to a family of protein dimers that share a common β-chain, one of which is the C3bi-receptor (13, 37) involved in phagocytosis of C3bi-coated particles by macrophages (10). A similarity with LFA-1 is that this function of the receptor is not simply dependent on its presence: phagocytosis occurs only when macrophages are activated (10). Furthermore, like LFA-1-dependent adhesion in lymphoblastoid cells and macrophages, phagocytosis can be induced by TPA (48). This similarity suggests that both the C3bi-receptor and LFA-1 interact with the cytoskeleton, and that this interaction is directly or indirectly regulated by protein kinase C. Further support for this notion is the phagocytosis of anti-LFA-1-coated liposomes by lymphocytes (46). Since cytoskeletal activity requires physiological temperatures, this suggestion would explain why LFA-1-dependent adhesion does not occur in the cold in contrast to CD2- and LFA-3-dependent adhesion (39). An attractive possibility is that binding of LFA-1 to its counterstructure induces the spreading of lymphoma cells on the hepatocyte upper surface, thus increasing the surface area and hence the strength of adhesion. This possibility is now being studied.

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