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A Cluster of Antibodies (RR1/1, LB-2 and 84H10) That Inhibit LFA-2-Dependent Lymphoid and Myeloid Cell Adhesion Bind Intercellular Adhesion Molecule-1 (ICAM-1)

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Abstract: Recent functional studies indicate that intercellular adhesion molecule-1 (ICAM-1), recognized by monoclonal antibody (MAb) RR1/1, is a ligand for lymphocyte-associated antigen (LFA)-1 in several models of antigen-independent B- and T-cell adhesion and in cytotoxic T-cell recognition. Independent reports have identified other MAbs (LB-2 and 84H10) that inhibit LFA-1-dependent adhesion by B cells or myeloid cells. We analyzed the relationships of these three MAbs. The results show that: 1) all three MAbs inhibit LFA-1-dependent conjugate formation with particular targets and that mixes of these MAbs do not show additive inhibition; 2) LB-2 and 84H10 each inhibit the binding of the other, whereas binding of RR1/1 neither inhibits nor is inhibited by binding of LB-2 or 84H10; 3) all three MAbs bind purified 84H10 protein; and 4) binding of these three MAbs is concordant on 12 different cell types and is similarly enhanced on monocytes with activation. The simplest interpretation of these data is that a single protein, ICAM-1, is recognized by the MAbs RR1/1, LB-2, and 84H10. This finding unifies and extends previous observations; ICAM-1 functions as an adhesion molecule in a variety of leukocyte interactions, apparently as a ligand for LFA-1. Furthermore,

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Table 1. MAb Inhibition of LFA-1-Dependent Conjugate Formation

Mab	HeLa	U937	U266
TS2/9	38	24	27
MHM23	0	2	2
LB-2	ND	1	23
RR1/1	2	2	21
84H10	3	1	23
84H10 + LB-2	ND	1	24
84H10 + RR1/1	2	4	21
RR1/1 + LB-2	ND	2	24

Conjugate formation was measured between the cytotoxic T-cell clone 8.2 and three antigen-negative targets HeLa, U937, and U266. Enumeration of conjugates were by two-color microfluorometry as described (10,19). The results are expressed as a % of targets that end up in conjugates. All assays were done in the continuous presence of saturating concentrations of the LFA-3 MAb TS2/9(17) to isolate the LFA-1 pathway. MAbs were continuously present during the assay at 100 µg/ml purified IgG except for MHM23, the LFA-1 Mab (20) and TS2/9 which were present at 300 µg/ml of Fab fragment. ND = not determined.

Table 2. MAb Binding to Purified ICAM-1 Protein

MAb	OD
TS2/9	75
84H10	850
LB-2	750
RR1/1	800

ICAM-1 was purified from L428 cells by an 84H10 immunochromatography column as described (11). The purified material was applied to glutaraldehyde/poly-L-lysine treated microtiter plates as described before (11). MAbs binding to the immobilized antigen was measured by ELISA using purified MAb (IgG) at 50 µg/ml as the first step, β-galactosidase-conjugated $F(ab')_2$ sheep anti-mouse IgG (H+L) as the second step, and measuring the rate of conversion of p-nitrophenyl-β-galactoside to a colored product (using the Bethesda Research Laboratories Hybridoma Screening Reagent according to the manufacturers directions). Data are reported as the average of the OD 405 nm of duplicate wells after subtraction of the OD in the absence of first step MAb.

MAb binding to either of two distinct ICAM-1 epitopes inhibits ICAM-1-dependent adhesion.

The LFA-1 antigen was identified 5 years ago in studies of monoclonal inhibition of antigen-specific cell-mediated cytotoxicity in both man and mouse (1,2). It has since been shown to be an adhesion receptor for all types of leucocytes (3,4) in a variety of such interactions. LFA-1 is a member of the cytoadhesin family of adhesion receptors (5–8). Homology between the members of this family extend to the position-specific protein (PSP) found in *Drosophila* (7,8), indicating that the cytoadhesins form a primitive cell-cell recognition system in both mammalian and nonmammalian species. Intercellular adhesion molecule-1 (ICAM-1) was postulated as a ligand for LFA-1 based on studies of monoclonal inhibition of homotypic B-cell aggregation and heterotypic adhesion of T lymphoblasts to fibroblasts (9,10). These findings have been extended and confirmed in studies of antigen-independent conjugate formation with T-cell clones (11). Recently, the ICAM-1 monoclonal antibody RR1/1 was shown to inhibit certain but not all types of antigen-specific CML interaction, raising the possibility of other alternative molecules that may act as LFA-1 ligands (12,13). Other MAbs had been described that inhibited leukocyte adhesion in a variety of models systems and suggested that they may act as ligands. Are these alternative LFA-1 ligands? The two relevant MAbs are 84H10 and LB-2. The MAb 84H10 was identified by preferential binding to myeloid leukemic cells and subsequently found to inhibit such cells to bone marrow stromal cells (unpublished observation). More recently, the 84H10 MAb has been shown to inhibit LFA-1-dependent homotypic B-cell aggregates (14). The MAb LY-2 was identified as a B-cell activation antigen (15) and subsequently shown to inhibit both myeloid and B-cell homotypic aggregates (16).

The initial studies compared the ability of three MAbs (RR1/1, LB-2, and 84H10) to inhibit LFA-1-dependent antigen-independent conjugate formation between a T-cell clone and targets of three different cell lineages. The LFA-1 pathway of adhesion was isolated by complete inhibition of the CD2/LFA-3 pathway using saturating amounts of the LFA-3 mab TS2/9 Fab (17). The results (Table 1) demonstrated a similar degree and pattern of inhibition by all three MAbs on the three targets. Furthermore, there was no additive inhibition when mixes were used as compared with single MAbs alone. The result suggested that the three MAbs act in the same pathway, either by binding to the same epitope or to different epitopes. The lack of inhibition on the target U266 raised the possibility of alternative molecules that may act as LFA-1 ligands, as has been previously suggested (9,12,13).

To determine whether all three MAbs recognize the same antigen, binding studies were carried on an 84H10 immunopurified protein bound to microtiter plates by the gluteraldehyde/poly-L-lysine method (11). The immunopurified 84H10 protein from L428 cells ran as a single band of 95 kD on SDS-PAGE (11). All three MAbs bound to the purified ICAM-1 (Table 2). The specificity of binding was demonstrated by the lack of binding of an irrelevant LFA-3 MAb TS2/9. This finding unifies a number of observations regarding the function, structure, and genetics of ICAM-1 (see below).

Because these three MAbs bind ICAM-1 and inhibit LFA-1-dependent adhesion, it was evaluated whether they bind to the same epitope. LB-2, 84H10, and RR1/1 MAbs were radiolabeled and the binding of each to L428 cells was measured in the presence of an excess of each MAb (Fig. 1). LB-2 and 84H10 appear to bind to the same epitope since they compete with each other for binding. RR1/1 binds to a different epitope since no

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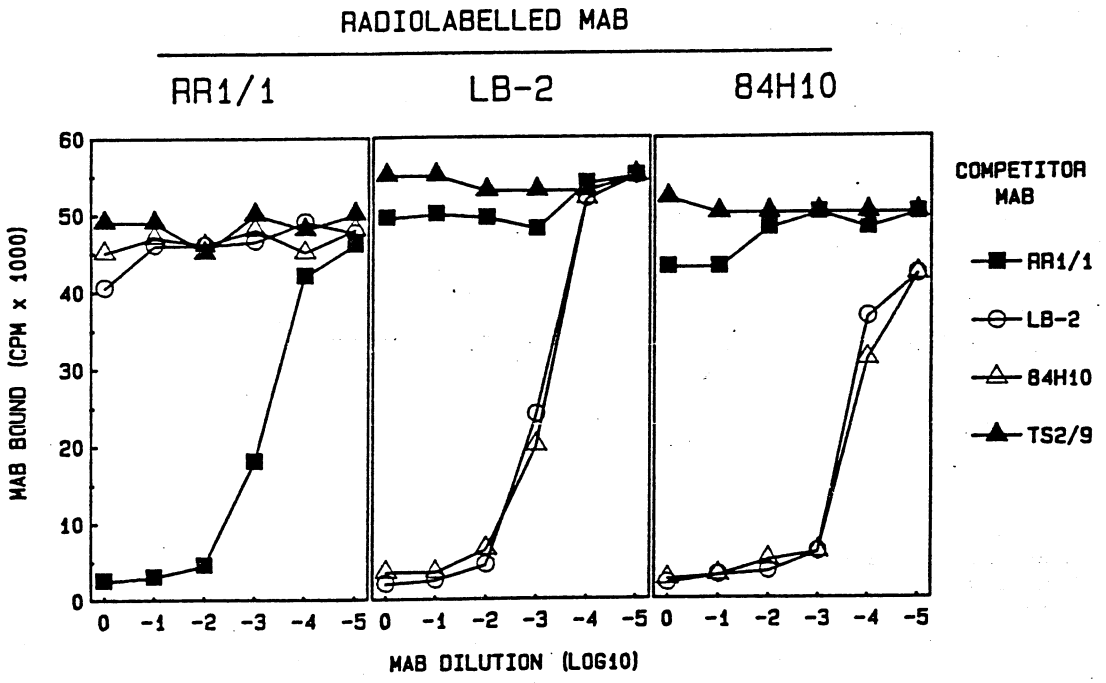

Figure 1. Competitive binding assay. MAbs were radiolabeled with ^{125}I by the iodo-bead method according to the manufacturer's protocol (Pierce Chemical Co., Rockford, IL). Usually 50% of the MAb retained activity by serial absorption studies. Serial 10-fold dilutions of the unlabeled MAbs were prepared in 0.5% human serum albumen in phosphate buffered saline starting at a concentration of 5 mg/ml. L428 cells at 2×10^5 per tube in 100 µl of 0.5% human serum albumen/phosphate buffered saline were preincubated with 30 µl of unlabeled MAb at 4°C for 15 minutes. After this, 20 µl of radiolabeled MAb equivalent to 0.1 µg was added and further incubated for another 60 minutes. The samples were washed three times with buffer and counted for radioactivity.

inhibition was seen in either direction between LB-2 or 84H10 and RR1/1. No nonspecific inhibition was observed since the TS2/9 MAb which binds to LFA-3 did not inhibit the binding of radiolabeled LB-2, 84H10, and RR1/1. Thus, ICAM-1 MAb that inhibit LFA-1-dependent adhesion can do so by binding to at least two distinct epitopes on ICAM-1.

If all three MAbs bind the same protein, a simple testable hypothesis is to study the tissue distribution of each epitope with the prediction that their expression would be concordant. Our studies using 12 different cell types show an identical pattern for the RR1/1 and 84H10/LB-2 epitopes (Table 3). Other studies demonstrated staining of a variety of cells on tissue sections, particularly endothelium for RR1/1 epitope (9,10). This pattern of staining is consistent with that reported for the 84H10 epitope in the Third International Workshop on Leukocyte Differentiation Antigens (18) or expression of the LB-2 epitope (16). Binding is coordinately enhanced for both epitopes following activation of monocytes (MWM in preparation).

The above studies demonstrate that the molecules defined by the MAbs LB-2, 84H10 are identical to the antigen ICAM-1 defined by the MAb RR1/1. There is

Table 3

(author please supply)

variation between cell types in ICAM-1 molecular weight as described for RR1/1 ranging from 90 to 114 kD (9), LB-2 antigen of 76 kD (15), or 84 kD (16), and the 84H10 antigen of 105 kD (18). This finding is consistent with the interpretation that cell-type-dependent differences in carbohydrate processing account for the variation in molecular weight. With regard to genetics, the gene coding for ICAM-1 maps to chromosome 19 based on prior studies with the LB-2 MAb (15). Finally, studies of cell adhesion using purified ICAM-1 have shown that ICAM-1 mediates LFA-1 dependent adhesion of B, T, and myeloid cells (11,12). The adhesion is inhibited by each of the three ICAM-1 MAbs and the LFA-1 MAb (11). This provides biochemical evidence that ICAM-1 is a ligand for LFA-1 in a variety of leukocyte interactions, and furthermore, that adhesion can be inhibited by each of two distinct epitopes on the ICAM-1 protein.

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Table 3.
Expression of the RR1/1 and 84H10/LB-2 epitopes on Human cells and cell lines.

Cell Type	Description.	RR1/1	84H10
F2B	B cell line	813	1076
DAUDI	Burkitt's lymphoma	918	820
U266	Plasmacytoid	2066	1323
Jurkat	T cell line	64	64
8.2	T cell clone	224	189
PBT	Peripheral Blood T cells	4	4
PBA	Peripheral blood adherent cell	483	288
U937	Promomocytoid cell line	205	175
L428	Reed-Sternberg cell line.	2441	3094
K562	Erythroleukaemia cell line	672	577
RBC	Human erythrocytes	0	0
HeLa	Cervical carcinoma cell line	700	1258

Expression of the RR1/1 and 84H10 epitopes was measured by flow microfluorometry as described (21) on 12 indicated cell types. Data were collected on 50,000 cells by using a logarithmic gain. Flourescence intensity is expressed in the linear units of millivolts. Expression on all cell types was unimodal. Values above 5 mv are positive.