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ON THE SPECIES SPECIFICITY OF THE INTERACTION OF LFA-1 WITH INTERCELLULAR ADHESION MOLECULES[1]

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Species restrictions in immune cell interactions have been demonstrated both in Ag-specific responses of T lymphocytes and the phenomenon of natural attachment. To determine the possible contribution of adhesion receptors to these restrictions, we have studied binding between the murine and human homologues of LFA-1 (CD11a/CD18) and ICAM employing purified human LFA-1 and ICAM-1 (CD54) bound to solid substrates. Murine cell lines bind to purified human LFA-1 through ICAM-1 and at least one other counter-receptor. This provides evidence for multiple counter-receptors for LFA-1 in the mouse as well as in the human. In contrast to binding of murine ICAM-1 to human LFA-1, murine LFA-1 does not bind to human ICAM-1. The species specificity maps to the LFA-1 α subunit, because mouse × human hybrid cells expressing the human α subunit associated with a mouse β subunit bind to human ICAM-1, whereas those with a human β subunit associated with a murine α subunit do not. Increased adhesiveness for ICAM-1 stimulated by phorbol esters could be demonstrated for hybrid LFA-1 molecules with human α and murine β subunits.

T lymphocyte responses are generally much more efficient within a species than across species, as shown 20 years ago with mixed lymphocyte reactions (1, 2). More recently, these findings have been confirmed in studies of Ag-specific responses to cells transfected with foreign MHC molecules (3–11). Inefficiency in recognition by human T cells of human MHC molecules transfected into mouse cells could be caused either by poor recognition of the MHC molecule because of its association with different peptides endogenous to mouse or human cells, or to poor interactions between human adhesion receptors on the T cell and the murine counter-receptors on the target cells. The latter hypothesis is supported by studies of Ag-independent adhesion between T lymphocytes and a variety of cell types, a phenomenon termed "natural attachment," in which binding appears to have a species-specific component (12, 13).

LFA-1[3] and ICAM molecules are receptors for one another that promote cell adhesion and facilitate Ag-dependent and Ag-independent T lymphocyte functions (14–16). The integral role of LFA-1/ICAM adhesion in Ag-specific responses has been demonstrated within the human system in studies showing that mAb to either LFA-1 or ICAM-1 blocks allogeneic CTL-mediated killing and proliferation of T cells in response to Ag (14, 15, 17, 18). Ag-independent responses mediated by LFA-1 interaction with ICAM-1 include phorbol-ester-stimulated homotypic adhesion (19) and lymphocyte adhesion to fibroblasts (20), endothelial cells (21), and keratinocytes (22). Human leukocytes have been shown to bind through LFA-1 to purified ICAM-1 on artificial substrates (23, 24), and through ICAM to purified LFA-1 (25). Furthermore, purified micellar LFA-1 binds to purified ICAM-1 on a substrate (25).

LFA-1, a cell-surface glycoprotein formed by the association of a 180-kDa α subunit (CD11a) and a 95-kDa β subunit (CD18) (26, 27), was originally identified in the mouse (28) and later in the human system where it has been cloned and sequenced (29, 30). It is a member of a family of leukocyte integrins, including Mac-1 and p150,95, that share the same β subunit. ICAM-1 and ICAM-2 are cell-surface glycoproteins of the Ig superfamily, which were first identified in the human as counter-receptors for LFA-1 (19, 31). A murine cell-surface Ag identified by the mAb YN1/1.7 and noted to inhibit allogeneic T lymphocyte responses (32) has recently been demonstrated to be the murine homologue of ICAM-1 based on its amino acid sequence (33–35).

In order to clarify the role of LFA-1/ICAM interactions in cellular adhesion between species, and to begin to answer questions about the regions of the LFA-1 and ICAM molecules that are important to their adhesive function, we have studied binding of human and murine cells to purified human LFA-1 and ICAM-1. Cross-species binding is unidirectional, occurring only between human LFA-1 and murine ICAM, and provides evidence for another murine ICAM in addition to ICAM-1. Assays with mouse × human hybrid lymphocytes show that the inter-

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[3] Abbreviations used in this paper: LFA-1, lymphocyte function-associated Ag 1; ICAM, intercellular adhesion molecule; TS, 25 mM Tris-HCl and 150 mM NaCl, pH 8.0, at 4°C; OG, octyl glucopyranoside; MxBWα[+] and MxBWβ[+], mouse-human hybrids expressing either the human LFA-1 α or β subunit.

action between mouse LFA-1 and human ICAM-1 is restricted by the murine α subunit.

MATERIALS AND METHODS

Cells. SKW-3, a human T lymphoma, was obtained from Peter Cresswell, Duke University, Durham, NC. BW5147 and EL-4 are mouse T lymphomas (36, 37), Ch-1 is a mouse B lymphoma (38), and NS-1 is a mouse myeloma (39). All four can be obtained from American Type Culture Collection, Rockville, MD. MxBWα^+ and MxBWβ^+ were previously developed and characterized (40). Briefly, PHA blasts were prepared from normal PBMC, fused with murine hypoxanthine-guanine-phosphoribosyl-transferase-deficient BW5147 cells, selected in hypoxanthine-aminopterin-thymidine media, and sorted for human LFA-1 subunit expression by flow microfluorometry. All cells were maintained in RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 μg/ml gentamicin, and 20% FCS (complete medium).

mAb. All mAb used have been previously described. TS1/18 and TS1/22 are blocking mouse IgG1 specific for human LFA-1 α and β subunits, respectively (27). M7/14 is a rat mAb specific for murine LFA-1 α subunit (28). M18/2 and C71/16 are rat mAb specific for murine LFA-1 β subunit (41, 42). RR1/1 and R6.5 are mouse IgG1 specific for human ICAM-1 (19, 43). YN1/1.7 is a rat IgG2a that binds to murine ICAM-1 and blocks MLR (32, 33). PA3 is a rat IgM that binds to a murine 55-kDa cell-surface glycoprotein and blocks T cell responses to Ag/MHC presented by B cells and macrophages (44). W6/32 is a mouse mAb specific for human HLA-A,B (45), and M1/42 is a rat IgG2a specific for murine MHC class I (46). R6.5 Fab fragments were a gift of Dr. R. Rothlein, Boehringer Ingelheim, Ridgefield, CT.

Protein-coated plates. Human ICAM-1 was purified as previously described (23). With the use of the same cell lysate, LFA-1 was purified by immunoaffinity chromatography on TS2/4 mAb-Sepharose. LFA-1 was eluted with 50 mM triethylamine, pH 11.5, 150 mM NaCl, 2 mM $MgCl_2$, 1% OG and neutralized immediately with 1 M Tris-HCl, pH 7, 2 mM $MgCl_2$, 1% OG (M. L. Dustin and T. A. Springer, manuscript in preparation). For absorbing LFA-1 or ICAM-1 to plates, 5 μl of protein solution at approximately 35 μg/ml in TS with 1% OG and 2 mM $MgCl_2$ or an identical buffer without protein was combined with 45 μl of 1 mM $MgCl_2$ in TS and incubated for 12 h at 4°C in wells of a flat bottomed, 96-well plate (Linbro/Titertek, Flow Laboratories, McLean, VA). Plates with varying LFA-1 densities were made in a similar manner by using different concentrations of protein solution in the wells. To block possible binding sites on the polystyrene, wells were washed and incubated for 1 h in TS with 1 mM $MgCl_2$ and either 5% nonfat dry milk (Carnation, Los Angeles, CA; for studies involving BW5147 and hybrid cell binding) or 1% BSA (when only SKW-3, Ch-1, and EL-4 were used). Wells were washed three times in appropriate medium before use.

Binding assay. Cells were radioactively labeled by incubating in 400 μCi/ml $Na_2{}^{51}CrO_4$ at 37°C for 1.5 h with gentle agitation. All subsequent washes and incubations were performed in complete medium with 25 mM HEPES, pH 7.4, for assays involving only Ch-1, EL-4, and SKW-3, and in RPMI 1640 with 25 mM HEPES, pH 7.4, and 0.5% nonfat dry milk for assays of BW5147, MxBWα^+, and MxBWβ^+ binding. After three washes, viability was checked by trypan blue exlusion (always greater than 95%), and cells were counted and diluted to give 4×10^5 cells/ml. Antibody incubations of plates and cells were performed at 4°C for 30 min with mAb at concentrations of 1/500 ascites or 1/5 cell culture supernatant, or with 100 μg/ml purified Fab fragment. Flat bottomed, 96-well plates (Linbro/Titertek, Flow Laboratories) with adhered protein (prepared as described above) were preincubated with 50 μl/well of these solutions to which were added 50 μl/well of preincubated cell solution and 50 ng/ml PMA where indicated. Plates were spun gently at $20 \times g$, 4°C, 5 min to settle the cells, then incubated for 10 min at 37°C. After assuring that homotypic aggregation was insignificant by microscopy, wells were washed either by aspirating with an 18-gauge needle and replacing with medium for four cycles (aspiration wash), or by immersing the plates upside-down in 10 mM Na_2HPO_4, 150 mM NaCl, pH 7.4 (PBS), with 1 mM $MgCl_2$ and 0.5% BSA for 1 h at 24°C (gravity wash). Cells were removed from wells by incubating with 20 mM EDTA in PBS for 10 min at 37°C and subjected to γ counting. Percent of cells binding was calculated from the following equation: % binding = (experimental cpm/input cpm) × 100.

Ag site density. Purified TS1/18, specific for human LFA-1, was labeled by using 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (Pierce, Rockland, IL) (47). Wells were incubated in 20 μg/ml ^{125}I-labeled mAb for 2 h at 4°C with shaking, then washed six times by aspiration with a 23-gauge needle. Antibody was released by a 5-min incubation in 0.1 N NaOH and subjected to γ counting. By using the specific activity of the antibody, the number of antibody-binding sites/square micrometer was calculated.

Homotypic aggregation assay. This assay was based on one previously described (48). Cells were washed three times and diluted in complete medium with 25 mM HEPES to give a final concentration of 2×10^6 cells/ml. In flat bottomed, 96-well plates (Linbro/Titertek, Flow Laboratories), 100 μl of cell solution were combined with 50 ng/ml PMA and mAb at 1/400 ascites fluid or 1/4 tissue culture supernatant, or with 250 μg/ml Fab. After 2 h at 37°C and 5% CO_2, degree of aggregation was scored under a microscope as follows: 0 indicated that essentially no cells were clustered; 1 indicated that <10% of cells were aggregated; 2 indicated clustering of <50%; 3 indicated that up to 100% of cells were in small, loose aggregates; 4 indicated that nearly 100% of cells were in larger clusters; 5 indicated that nearly 100% of cells were in very large, tight clusters.

RESULTS

Murine cell binding to human LFA-1. Cell clustering occurs in several different leukocyte lines after activation with phorbol esters such as PMA. This homotypic aggregation has been shown to occur primarily through LFA-1/ICAM interaction (19, 49). Homotypic aggregation assays were performed to assure that functional LFA-1 and ICAM were expressed on the murine lymphocyte lines characterized below for ability to bind to purified human LFA-1 and ICAM-1. Both the Ch-1 B lymphoma and EL-4 T lymphoma cell lines aggregate profoundly in the presence of PMA, and this aggregation is completely inhibited by a mAb to murine LFA-1 (Table I), indicating that LFA-1 and at least one of its counter-receptors are present on the lines. The murine NS-1 myeloma line failed to aggregate under any of the conditions tested (not shown).

A mAb to murine ICAM-1 (33), YN1/1.7, which has been shown to block MLR (32), homotypic aggregation of mouse lymphoblasts (49), and LFA-1-dependent binding of murine lymphoblasts to purified murine ICAM-1 (33), does not significantly inhibit homotypic aggregation of Ch-1 and EL-4. PA3 mAb, which may recognize a murine counter-receptor for LFA-1 based on its specificity in inhibiting T lymphocyte responses, (44), also does not block homotypic adhesion. Furthermore, the combination of YN1/1.7 and PA3 is not inhibitory. Failure of YN1/1.7 and PA3 to block homotypic aggregation is not caused by poor mAb binding because flow cytometry showed that both mAb brightly stain Ch-1 and EL-4 (not shown).

To examine cross-species binding between human LFA-1 and murine counter-receptors, murine cell lines were tested for binding to purified human LFA-1 adhered to 96-well polystyrene plates. Both EL-4 and Ch-1 show significant binding to human LFA-1 (Fig. 1). No binding is observed when the wells with absorbed LFA-1 are treated with TS1/22, an antibody specific for human LFA-1, or when EDTA is added (data not shown), showing that the interaction depends on LFA-1 and on divalent

TABLE I
PMA-induced homotypic aggregation[a]

Antibody Pretreatment	Aggregation Score	
	Ch-1	EL-4
M1/42 (anti-H-2)	4	4
M7/14 (anti-LFA-1)	0	0
YN1/1.7 (anti-ICAM-1)	4	3
PA3	4	4
PA3 + YN1/1.7	4	3

[a] A murine B lymphoma (Ch-1) and T lymphoma (EL-4) were incubated in PMA and the indicated mAb and scored for homotypic aggregation.

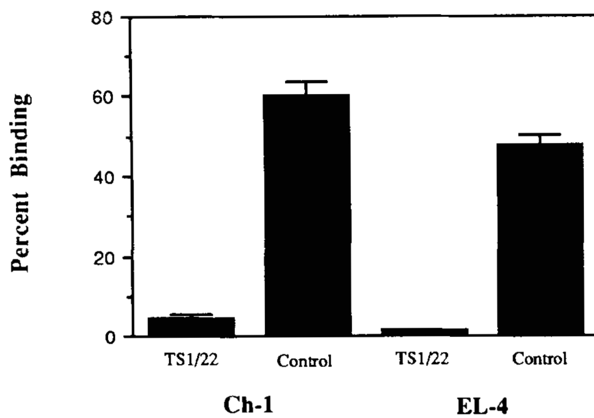

Figure 1. Murine lymphocytes bind to solid phase human LFA-1. Ch-1, a murine B lymphoma, and EL-4, a murine T lymphoma, were tested for binding to human LFA-1 absorbed to polystyrene in 96-well plates. Wells were preincubated with and without TS1/22, a mAb specific for human LFA-1. Results are expressed as the mean ± SD of quadruplicate determinations and are representative of two experiments.

cations. For all cell types and experimental conditions tried, less than 5% of input cells bind in control wells coated with buffer and BSA but lacking LFA-1.

The dependence of binding on LFA-1 site density was tested by varying the concentration of purified LFA-1 used in coating the wells. Binding is dependent on LFA-1 density, and murine Ch-1 B lymphoma (Fig. 2*A*), EL-4 T lymphoma (Fig. 2*B*), and NS-1 myeloma cells (Fig. 2*C*) have different binding patterns over a range of LFA-1 densities. Adhesion of all three lines is completely blocked in the presence of a mAb against human LFA-1. As in the homotypic aggregation assays, YN1/1.7 and PA3 mAb have no effect on binding of the Ch-1 and EL-4 lymphoma lines.

To determine whether YN1/1.7 mAb failed to block binding of the lymphoma lines to human LFA-1 because a counter-receptor other than ICAM-1 was involved or because the YN1/1.7 mAb was a poor inhibitor of the murine ICAM-1/human LFA-1 interaction, we tested the NS-1 murine myeloma, which has been shown to richly express ICAM-1 (32). NS-1 binding to human LFA-1 is nearly completely eliminated in the presence of YN1/1.7 mAb to murine ICAM-1 (Fig. 2*C*). This shows that murine ICAM-1 can bind to human LFA-1. The findings further suggest that another counter-receptor for human LFA-1, possibly ICAM-2, is expressed on the murine Ch-1 and EL-4 lymphomas because binding of these cell lines to LFA-1 is not blocked by YN1/1.7 mAb (Fig. 2*A* and *B*).

Murine cell binding to human ICAM-1. A reciprocal assay was used to study cross-species binding between human ICAM-1 and murine LFA-1. The two murine lymphoma lines, both expressing functional LFA-1 as shown by homotypic aggregation (Table I), were activated with phorbol ester (PMA) and tested for binding to purified human ICAM-1 absorbed to microtiter wells. Even with gentle washing conditions and PMA stimulation, there is negligible binding of the murine lymphocytes to purified human ICAM-1 on solid substrates (Fig. 3), showing that murine LFA-1 does not significantly bind to human ICAM-1. In the same assay, SKW-3 human T lymphoma cells bind human ICAM-1, and the degree of binding is profoundly enhanced by treatment with PMA. SKW-3 binding is blocked by anti-ICAM-1 Fab fragments but Ch-1 and EL-4 binding is unchanged (not shown), suggesting

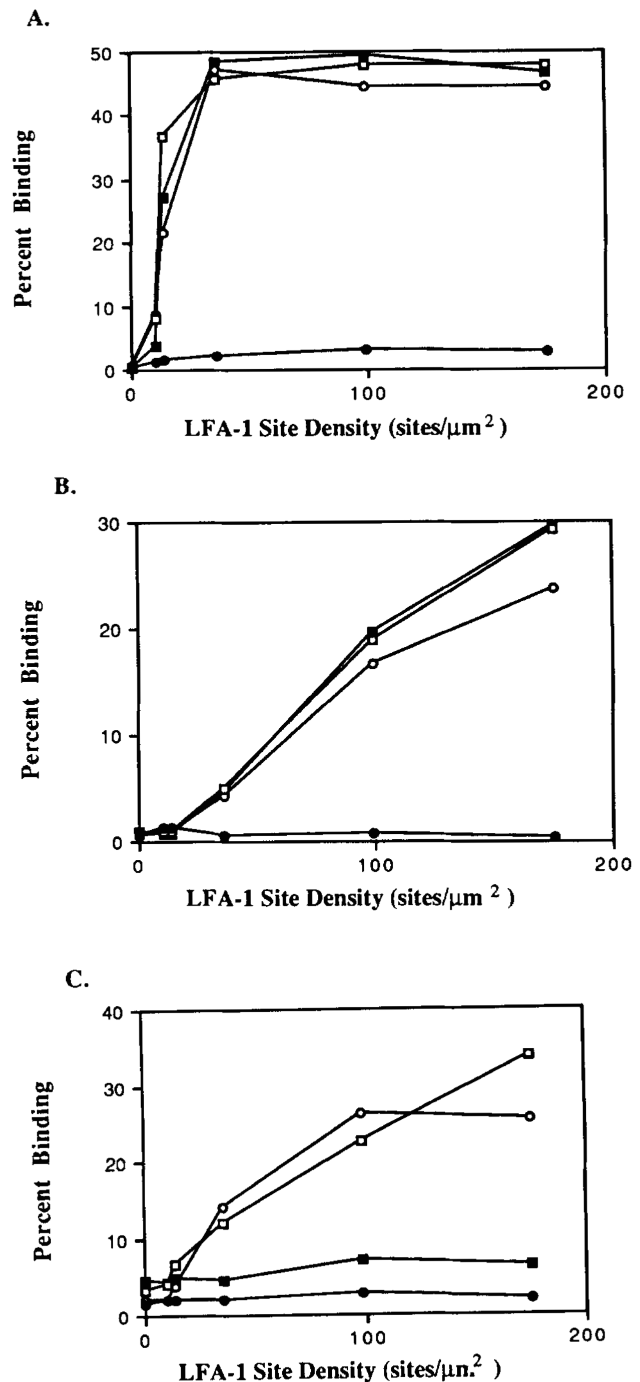

Figure 2. Murine cell binding to human LFA-1 is dependent on LFA-1 site density and, for a myeloma line, can be blocked by antibody to murine ICAM-1. *A*, Ch-1, a B lymphoma; *B*, EL-4, a T lymphoma; and *C*, NS-1, a myeloma. Binding assays were performed over a range of human LFA-1 concentrations absorbed to polystyrene in 96-well plates. LFA-1 site density was determined by RIA. Cells and wells were preincubated with mAb to human LFA-1 (TS1/22, *closed circles*), with mAb to murine ICAM-1 (YN1/1.7, *closed squares*), with PA3 mAb (*open squares*), or with a control mAb to murine MHC class I (M1/42, *open circles*). Results are expressed as the mean of quadruplicate determinations. SD were consistently less than 5% of input.

that the small degree of murine cell binding is not caused by LFA-1/ICAM interactions.

Hybrid cell binding to human ICAM-1. To determine whether one of the human LFA-1 subunits in association with the complementary murine subunit would allow binding to human ICAM-1, mouse-human hybrids expressing both murine LFA-1 subunits and either the

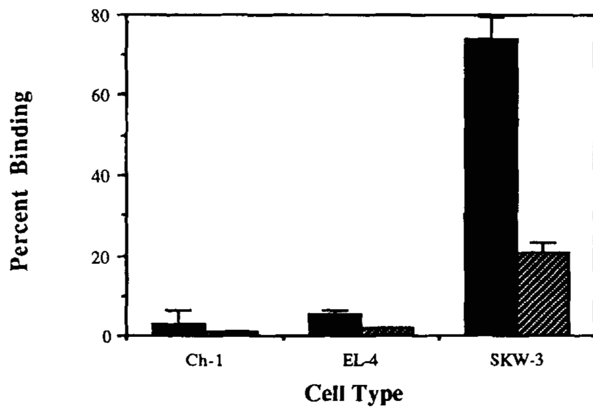

Figure 3. Murine lymphocytes do not bind to solid phase human ICAM-1 even after PMA stimulation. A human T lymphoma (SKW-3) and murine B cell (Ch-1) and a T cell (EL-4) lymphoma line were tested for binding to human ICAM-1. Cells were incubated for 10 min with (*black*) or without (*hatched*) PMA. Results are expressed as the mean ± SD of quadruplicate determinations and are representative of two experiments.

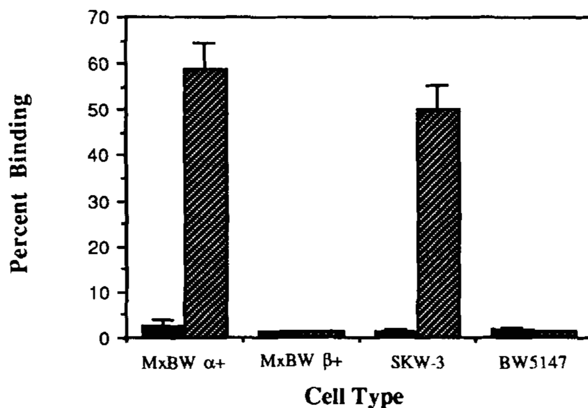

Figure 4. Mouse-human hybrids expressing the human LFA-1 α subunit (MxBWα^+) bind to human ICAM-1 but hybrids with only the human β subunit (MxBWβ^+) do not. Cells activated by incubation with PMA were tested for binding to human ICAM-1 absorbed to polystyrene in 96-well plates. Cells and wells were preincubated with either R6.5 Fab fragments specific for human ICAM-1 (*black*) or with a control antibody (*hatched*) to mouse MHC class I for murine cells and hybrids (M1/42 mAb) or to human MHC class I for human cells (W6/32). Results are expressed as the mean ± SD of quadruplicate determinations and are representative of two experiments.

human LFA-1 α subunit or β subunit (40) were tested. The hybrid cell lines were checked by flow cytometry for murine and human LFA-1 subunit expression before use to assure that only one of the human subunits was present and that it was expressed in comparable amount to its murine equivalent (data not shown). Immunoprecipitation had previously shown human LFA-1 subunits to be associated with complementary murine subunits on the hybrid lines (40). The hybrid lines coexpress the murine α, murine β LFA-1 complex.

The ability of PMA-activated hybrid cells to bind purified human ICAM-1 was tested (Fig. 4). The hybrid line expressing the human α subunit (MxBWα^+) shows binding comparable to the human SKW-3 lymphoma line, but a similar cell line expressing the human β subunit (MxBWβ^+) does not bind. The LFA-1$^+$ murine cell line used for hybridization (BW5147) shows no significant adhesion, and none of the cell lines bind in the presence of a blocking Fab fragment specific for human ICAM-1 (R6.5). This suggests that expression of the human LFA-1 α subunit on the hybrids is adequate for adhesion to human ICAM-1.

Incubation with PMA enhances binding of MxBWα^+ and of a control human line (SKW-3) to a similar degree (Fig. 5), indicating that stimulation by phorbol ester of adhesion dependent on LFA-1, an effect previously identified in human and murine systems (48, 50, 51), is conserved in the hybrid cells expressing the human α subunit, and suggesting further functional similarities between human LFA-1 and the human α, murine β LFA-1 complex.

To confirm the importance of the human α subunit and not the β subunit in hybrid cell binding to human LFA-1, the ability of various LFA-1 mAb to block adhesion was tested. An antibody to murine LFA-1 α subunit (M7/14), which has been shown to block CTL-mediated killing, Ag-specific T cell proliferation, and MLR (28, 52), does not block binding of MxBWα^+ but an antibody to the human α subunit (TS1/22) does (Fig. 6), confirming involvement of human CD11a in MxBWα^+ binding to human ICAM-1 (Fig. 6). A mAb to the human β subunit (TS1/18) blocks binding of human SKW-3 lymphoma

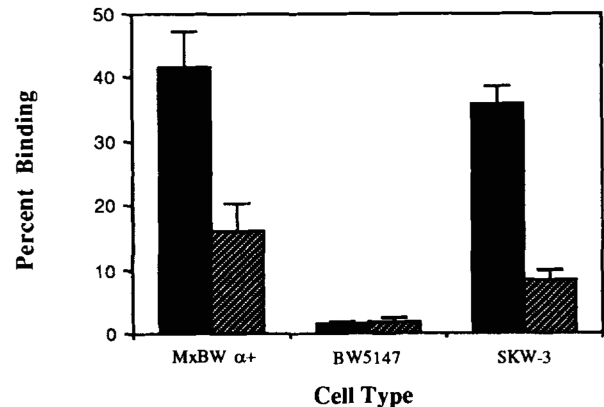

Figure 5. Binding of mouse × human hybrid cells expressing human LFA-1 α subunit (MxBWα^+) to human ICAM-1 is enhanced after PMA activation. Cells were incubated for 10 min with (*black*) or without (*hatched*) PMA. Results are expressed as the mean ± SD of quadruplicate determinations and are representative of two experiments.

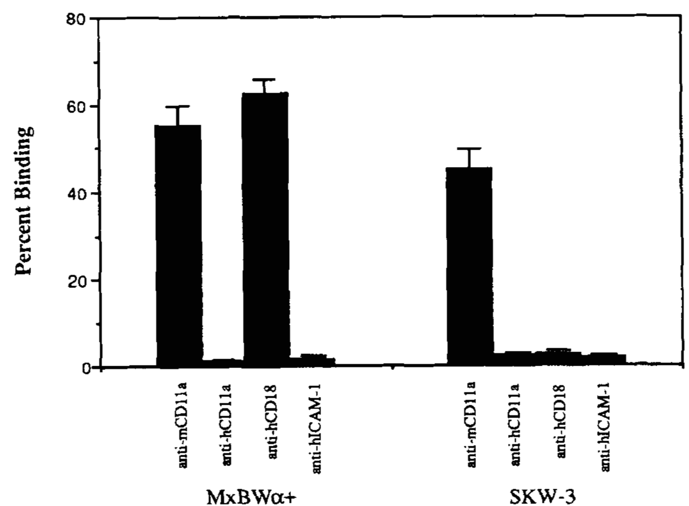

Figure 6. Identity of the LFA-1 subunits involved in binding of mouse × human hybrids to human ICAM-1. Cells were tested for binding to human ICAM-1 absorbed to polystyrene in 96-well plates. All cell types were activated by incubation with PMA. Cells and wells were preincubated with mAb against murine CD11a (anti-mCD11a, M7/14), human CD11a (anti-hCD11a, TS1/22), or human CD18 (anti-hCD18, TS1/18), or with Fab fragments specific for human ICAM-1 (R6.5, anti-hICAM-1). Results are expressed as the mean ± SD of quadruplicate determinations and are representative of two experiments.

cells to ICAM-1, but does not affect binding of MxBWα^+ hybrid cells, further supporting the conclusion that the human LFA-1 α subunit expressed on these hybrids is truly isolated from the human β subunit. Two mAb specific for the murine β subunit, M18/2 and C71/16, do not significantly inhibit MxBWα^+ binding to human ICAM-1 (data not shown). M18/2 was not expected to block MxBWα^+ adhesion because, in contrast to mAb to the LFA-1 α subunit, it does not block T lymphocyte-mediated killing (41). C71/16 has been shown to block CTL lysis in one effector/target cell combination but not in another combination that is effectively blocked by a mAb to the LFA-1 α subunit (42).

DISCUSSION

To discern the contribution of the LFA-1 adhesion receptor and its counter-receptors to cross-species cellular adhesion, and to examine functional homologies between murine and human LFA-1 and ICAM, murine cell lines, and mouse × human hybrids were tested for binding to purified human LFA-1 and ICAM-1 on solid substrates. Our results show substantial binding of murine cells to human LFA-1 that is abrogated in the presence of mAb against human LFA-1. In addition, binding of the NS-1 murine myeloma line is inhibited by an anti-murine ICAM-1 mAb, indicating that its adhesion to human LFA-1 is dependent on an interaction of murine ICAM-1 with human LFA-1. On the other hand, binding to two murine lymphoma lines, Ch-1 and EL-4, cannot be blocked by anti-murine ICAM-1, demonstrating that adhesion of these cell lines is mediated by a counter-receptor for LFA-1 that is distinct from ICAM-1. Whether this counter-receptor is equivalent to human ICAM-2 remains to be determined because it is possible that counter-receptors for LFA-1 in addition to ICAM-1 and ICAM-2 may exist. A mAb, PA3, that blocks Ag-specific murine T cell responses in a similar pattern to anti-LFA-1 but reacts with a molecule of 55 kDa distinct in size from both LFA-1 and ICAM-1 (44) failed to block binding to LFA-1 of any of the murine cell types tested. This negative result cannot be considered evidence for or against the possible identity of this molecule as a murine LFA-1 counter-receptor. Recently, our finding of adhesion between murine ICAM-1 and human LFA-1 has been confirmed in studies in this lab showing that COS cells transfected with murine ICAM-1 bind human LFA-1 on solid substrates at a level comparable to human ICAM-1 transfectants, and that this binding can be inhibited by either anti-human LFA-1 or anti-murine ICAM-1 mAb (53).

Because both murine and human ICAM-1 bind to human LFA-1, it seems likely that active sites for binding to LFA-1 are confined to regions of sequence homology. Murine ICAM-1 has 50% amino acid identity with human ICAM-1 (33–35). Site-directed mutagenesis of human ICAM-1 has confirmed that amino acids important in the interaction with LFA-1 are conserved in murine ICAM-1 (53).

Human ICAM-1 on solid substrates was used to test cross-species binding to murine LFA-1. Even with PMA stimulation and gentle washing, no significant binding of LFA-1$^+$ murine cell lines was observed. The human ICAM-1 was functional because LFA-1$^+$ human cell lines showed excellent binding in parallel experiments. Thus, even though murine ICAM-1 binds to human LFA-1, human ICAM-1 does not bind to murine LFA-1. By using mouse × human hybrid lines that express either one or the other of the human LFA-1 subunits associated with the complementary murine subunit, we were able to determine which of the murine subunits was responsible for the species restriction in ICAM-1 binding or, stated inversely, which human subunit was required for binding to human ICAM-1. Mouse × human hybrids expressing the human LFA-1 α subunit will bind to human ICAM-1. These hybrid cells express an LFA-1 formed from human α and murine β subunits (40). Similar hybrids that express the human β subunit associated with the murine α subunit do not bind to human ICAM-1. Besides illustrating the essential role of the LFA-1 α subunit in binding to ICAM-1, these results point to significant structural homology between the human and murine β subunits because both can associate with the human α subunit and facilitate binding to human ICAM-1. Indeed, the human and murine β subunits are 82% identical in amino acid sequence (54).

Our results begin to clarify the role of adhesion receptors in cross-species T cell responses. Human LFA-1 will interact with murine ICAM-1 and another murine counter-receptor, whereas human ICAM-1 will not interact with murine LFA-1. In Ag-specific T lymphocyte responses, it is the LFA-1 on the T lymphocyte that is functionally important, even when the target cell is an LFA-1$^+$ leukocyte (55). Thus, the LFA-1/ICAM interaction should contribute when human T cells interact with murine target and APC expressing ICAM. Murine T lymphocyte interactions with human target cells should be less efficient, because murine LFA-1 cannot interact with human ICAM-1. Whether murine LFA-1 can interact with another human ICAM remains to be determined.

Interactions between human LFA-1 and murine ICAM-1 in SCID mice with reconstituted human immune systems (56) may facilitate the observed partial reconstruction of immune function in the models.

The unidirectional species restriction of LFA-1/ICAM-1 binding recommends reevaluation of results from experiments involving T cell responses between murine and human cells. Previously, studies of immune reactions between cells of different species have generated confusing results, with some investigators noting relatively small xenogeneic T cell responses and others observing robust inter-species reactions, sometimes exceeding allogeneic responses (2, 57). Early experiments demonstrated strong xenogeneic cytolytic responses of human lymphocytes sensitized to murine target cells (58). Cross-species LFA-1/ICAM interactions likely contribute to the CTL lysis observed in these studies, and may help to explain the strength of the T cell responses observed.

Of course, if murine target cells do not express LFA-1 counter-receptors, LFA-1 mediated adhesion will not contribute to cross-species human T cell responses. Most murine L cell fibroblast lines appear to lack functional expression of murine ICAM molecules, as implicated by failure of LFA-1 mAb to affect Ag presentation, and by lack of expression of ICAM-1 (34, 59). Furthermore, transfection of L cells with ICAM-1 improves their efficiency as APC (11, 34). This lack of expression of ICAM-1 on L cells may explain the common observation that human T lymphocyte responses are generally stronger to human cells than to murine L cells transfected with

human HLA (3–11, 60), and that human CTL do not conjugate with L cells (9). By contrast, our observation of human LFA-1 interaction with murine ICAM-1 appears to explain the observation of LFA-1-dependent conjugation of human T cells to the murine P815 mastocytoma cell line (9) because P815 cells are ICAM-1$^+$ (49). Contrary to the conclusion that L cells lack a counter-receptor for murine LFA-1, two groups have shown that lysis of HLA-transfected L cells by some human allospecific CTL clones can be blocked by mAb to human LFA-1 (7, 60). This is in agreement with our finding that human LFA-1 can bind to murine counter-receptors and suggests that some L cell lines express murine ICAM. In a wide range of cell types examined in the human, including fibroblasts, ICAM-1 is inducible with cytokines and LPS (20–22), and thus differences in culture conditions or endotoxin concentration in growth media, as well as differences among L cell sublines, may explain why some groups, but not others, find an LFA-1 counter-receptor on L cells.

Thymocytes from mouse and human have been found to bind to a wide variety of cell types within, but not across, species in a phenomenon called natural attachment (12, 13). The bidirectional restriction in natural attachment is incompatible with the unidirectional restriction in LFA-1/ICAM-1 interaction, and thus other adhesion mechanisms may be involved in this phenomenon.

We have defined the species specificity of the interactions between murine and human LFA-1 and ICAM. Our findings are useful in interpreting past studies on xenogeneic cell interactions and guiding future work on this subject. Evidence for multiple counter-receptors for LFA-1 has now been extended to a second species, the mouse, and should stimulate the search for further murine ICAM molecules. The observation that the α subunit of LFA-1 can restrict interaction with ICAM-1 across species provides further evidence that the LFA-1 α subunit binds to ICAM-1 and provides information for guiding further studies of structure/function relationships in this system.

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