Brief Definitive Report

MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS

VIII. Role of the LFA-1 Molecule

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A subset of T cell surface antigens have been shown to play a role in the response of T cells to target cells. These molecules do not seem to be part of the antigen (Ag)-specific, major histocompatibility complex (MHC)-restricted receptor on T cells since they do not vary between T cell clones, yet antibodies against these molecules very frequently inhibit responses of T cells to Ag/MHC (1–4). For example, the lymphocyte function–associated antigen-1 (LFA-1) seems to be active in a broad range of T cell functions with no relation to the specificity of the response (5, 6).

In murine systems, all reports so far of the effects of monoclonal antibody directed against LFA-1 on T cell function have analyzed the interaction of T cells with cells of leukocytic lineage. In this paper we present data which show that anti-LFA-1 does not inhibit the responses of T cell hybridomas to Ag presented by nonleukocytic fibroblast cells derived from the L cell line, nor to polyvalent anti–T cell receptor antibodies, although the responses of these same T cell hybridomas to Ag presented by leukocytic cells are sensitive to the antibody. These results imply that LFA-1 does not play an essential role in T cell activation, and that the molecule may interact with ligands on leukocytic target cells which are absent from L cells.

Materials and Methods

Culture Medium. Cells were cultured in medium prepared by the recipe of Mishell and Dutton (7), with the exception that the medium was supplemented before use with 10% nutrient cocktail and contained 10% fetal bovine serum, 5×10^{-5} M 2-mercaptoethanol, and 50 μg/ml gentamicin (Shering Corp., Kenilworth, NJ). Transfected fibroblast lines were cultured in the same medium with hypoxanthine, aminopterin, and thymidine (HAT) added to maintain selection for cells expressing the transfected genes.

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TABLE I
Monoclonal Antibodies Used in These Studies

Antibody	Specificity	Source	Reference
121/7.7	LFA-1	Culture SN	8
GK-1.5	L3T4	Culture SN	3
KJ12-98.15	Self-D^d receptor on T cell hybrid 3DT-52.5	Culture SN	9
KJ1-26.1	Receptor for OVA/I-A^d on T cell hybridoma, DO-11.10	Culture SN	9

Reagents. Keyhole limpet hemocyanin (KLH) and ovalbumin (OVA), obtained from Sigma Chemical Co., St. Louis, MO, were used as Ag in these experiments. Alkaline phosphatase–conjugatec' rabbit anti–rat immunoglobulin and *p*-nitrophenyl phosphate were also purchased from Sigma Chemical Co.

Monoclonal Antibodies. Monoclonal antibodies were prepared as cell-free culture supernatants (SN) of hybridoma c ures grown to a cell concentration of 10^6 cells/ml. A list of the monoclonal antibodies used in these experiments is given in Table I. Monoclonal antibody culture supernatants were used neat.

T Cell Hybridomas. T cell hybridomas for these experiments were prepared by fusing Ag/MHC-reactive T cell blasts to BW5147 by standard procedures (10). The properties of these T cell hybridomas have been described elsewhere (10).

Transfected Fibroblast Lines. Two different transfected fibroblast lines were used in these studies. Both are products of transfections of L cells, C3H-derived fibroblast tumor cells, made thymidine kinase negative (TK^-) to render them HAT sensitive. Transfections were done with plasmid constructs containing the genes of interest plus the thymidine kinase gene. The first cell line, CA14.11.14, was transfected with the genes for I-A^k (11). The second cell line, a generous gift of Dr. John Seidman (Harvard University, Cambridge, MA) was transfected with the genes for D^d (12).

Interleukin 2 (IL-2) Assay for T Cell Hybridoma Activity. T cell hybridomas' recognition of Ag/MHC or stimulation by polyvalent anti-receptor antibody was assayed by the secretion of IL-2 by these hybridomas (10). None of these hybridomas made detectable IL-2 in the absence of the appropriate Ag/MHC or other stimulus. To induce IL-2 production, 10^4 hybridoma T cells were incubated for 24 h with the appropriate Ag and 10^5 antigen-presenting cells (APC), or with anti-receptor antibodies coupled to Sepharose beads in 250-μl cultures. Ag concentrations were always 500–1,000 μg/ml.

Results are expressed as units per milliliter of IL-2 secreted by the T cell hybridomas under different conditions. 10 U/ml IL-2 was the minimum detectable using this assay. A20.2J, a BALB/c B cell lymphoma, and P815, a DBA/2 mastocytoma, were used to stimulate a D^d-specific T cell hybridoma; LK-4.5, a B cell hybridoma, was used to present Ag to I-A^k-restricted T cell hybridomas.

ELISA for Cell Surface Antigens. We assayed for the expression of cell surface antigens by the standard enzyme-linked immunosorbent assay (ELISA) (13). This assay was slightly modified to use a vacuum plate system from Millipore Corp. (Bedford, MA).

Results and Discussion

It has been reported previously that the L cell transfectant CA14-11.14 expresses the transfected gene product I-A^k in sufficient concentration to serve as a presenting cell to I-A^k-restricted, Ag-specific T cell hybridomas. This is illustrated in Fig. 1, which shows that two I-A^k-restricted T cell hybridomas, SKK-2.3 and SKK-9.11, respond to KLH plus the transfected L cell, although somewhat less well than to KLH plus an equal number of cells of the B cell hybridoma, LK-4.5. The blocking effects of anti-L3T4 were equivalent when using either presenting line together with the anti-L3T4–sensitive T cell hybridoma, SKK-2.3. A different result was observed using anti-LFA-1 as the blocking antibody. The anti-LFA-1–sensitive hybrid SKK-9.11 was effectively blocked

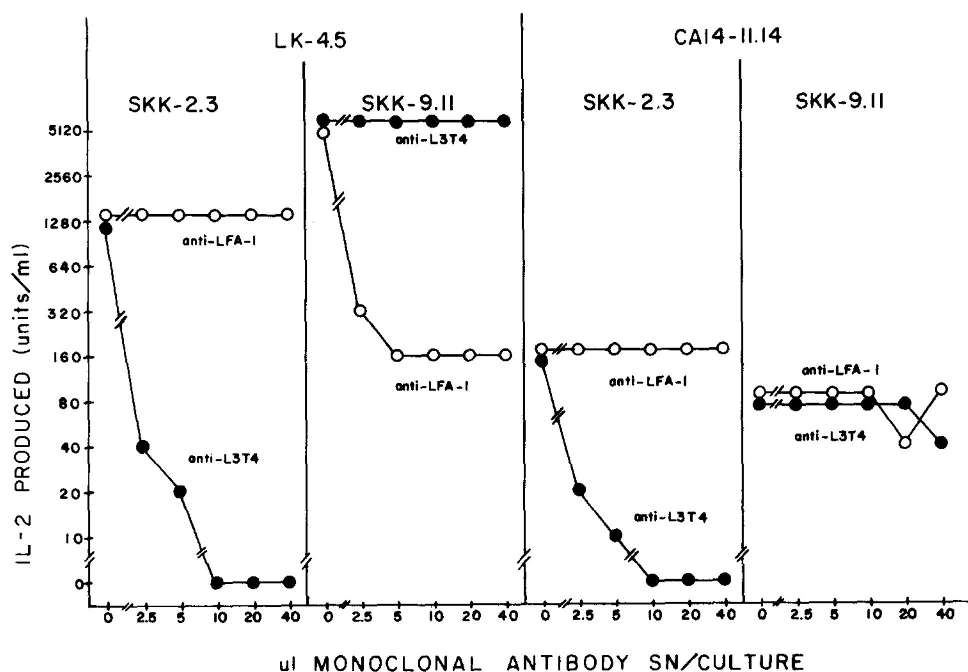

FIGURE 1. The response of a T cell hybridoma to KLH/I-A^k is unaffected by the blocking effects of anti-LFA-1 antibody when an I-A-transfected fibroblast is used as the presenting cell. The T cell hybridomas SKK-2.3 and SKK-9.11 were incubated with KLH plus the B cell hybridoma LK-4.5 or the I-A^k-transfected fibroblast line, CA14-11.14. Different concentrations of anti-L3T4 (●) or anti-LFA-1 (○) antibodies were added to these cultures. The effects of these antibodies on the responses of the T cell hybridomas were measured 24 h later by monitoring the concentrations of IL-2 secreted.

when LK-4.5 was the presenting cell line, but such blocking effects were lost when the CA14-11.14-transfected fibroblast was the presenting cell (Fig. 1).

The effects of anti-L3T4 on the responses of the T cell hybridomas, regardless of the presenting cell used, is consistent with the hypothesis that the target of L3T4 interaction is a nonpolymorphic determinant on the Ia molecule (14). The fact that anti-LFA-1 had no effect on the response of SKK-9.11 when L cells were used as presenting cells suggests that LFA-1 does not play a role in this interaction, discussed more fully below.

A similar result was seen when the D^d-specific T hybridoma, 3DT-52.5, was used (Fig. 2). This hybridoma responded comparably to the BALB/c B cell lymphoma A20.2J, the DBA/2 mastocytoma P815, and the D^d-transfected L cell line T4.8.3. KJ12-98.5, an antibody specific for the clonotypic receptor on 3DT-52.5, blocked the response of the T cell hybridoma to all of these cell lines comparably. However, the blocking effects of anti-LFA-1 were seen only when the two leukocytic cell lines, A20.2J and P815, were used as targets. No such blocking was observed in the response of 3DT-52.5 to T4.8.3.

The blocking effects of anti-LFA-1 do not seem to depend on expression of this glycoprotein by the APC themselves since, as shown in Table II, neither A20-2J nor the L cells and their transfectants, bore LFA-1. Thus anti-LFA-1 antibodies were able to inhibit Ag presentation by A20-2J to some T cell

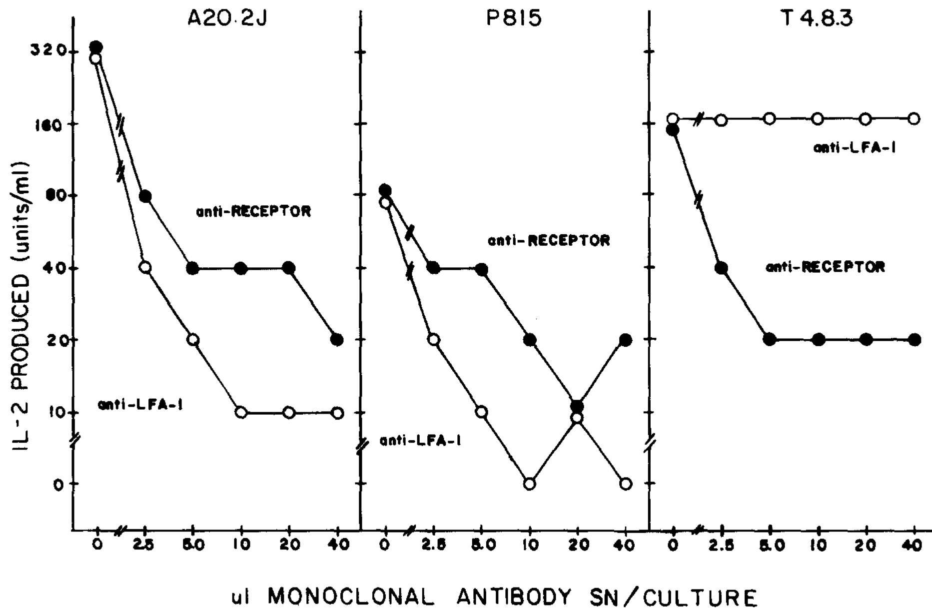

FIGURE 2. The response of a class I-specific T cell hybridoma is insensitive to the blocking effects of anti-LFA-1 monoclonal antibody when a transfected fibroblast is used as the stimulator. The T cell hybridoma, 3DT-52.5, was incubated with the D^d-presenting cell lines, A20-2J, P815, or T4.8.3 in the presence of various concentrations of anti-3DT-52.5 receptor antibody (●) or anti-LFA-1 (○). The effects of these antibodies on responses were measured 24 h later by monitoring concentrations of IL-2 secreted.

TABLE II

Expression of Cell Surface Antigens by These Cell Lines as Measured by ELISA

Cell line	ELISA OD × 10^3* after assay with 121/7.7 anti-LFA-1
3DT-52.5	661 ± 88
SKK-2.3	705 ± 45
SKK-9.11	392 ± 37
A20.2J	28 ± 37
P815	287 ± 42
LK-4.5	−3 ± 11
T4.8.3	−68 ± 29
L(TK⁻)	37 ± 67

* OD_{405} ± SE × 10^{-3}.

hybridomas, even though this cell line was LFA-1⁻. This result implies that LFA-1 inhibition acts on the LFA-1-bearing T cell hybridomas in these cultures, and argues against LFA-1 functioning in a like-like interaction.

It is difficult to account for the variations in the effects of anti-LFA-1 that depend on the APC in culture, given the conclusion that the primary targets of these antibodies are T cell hybridomas rather than APC. One explanation could be that the LFA-1 molecule is involved in the binding of some T cells to their targets, as an "accessory molecule" that interacts with an unknown ligand (not a

TABLE III
Anti-LFA-1 Does Not Inhibit the Response of T Cell Hybridomas to Polyvalent Anti-receptor Antibodies

T cell hybridoma	KJ1-26-Sepharose	IL-2 secreted in the presence of anti-LFA-1 SN at:					
		0*	2.5	5.0	10	20	40
		U/ml					
DO-11.10	OVA/A20-2J	320	40	20	10	10	<10
DO-11.10	KJ1-26-Sepharose beads	320	320	320	320	320	160
3DT-52.5	A20-2J	640	320	160	80	40	40
3DT-52.5	KJ1-26-Sepharose beads	160	160	160	160	160	160

* Microliters of anti-LFA-1 per 250-μl culture of SN from 121/7.7.

class I or class II molecule) on the target cells. If this explanation is correct, we would have to propose that LFA-1 is not involved in the interaction of T cells with L cells, perhaps because L cells do not bear the ligand for LFA-1.

If LFA-1 is simply acting as an accessory molecule in the binding of T cells and their targets, then the molecule should not play a role in the interactions of T cells with noncellular targets. To test this possibility, we challenged two T cell hybridomas, DO-11.10 and 3DT-52.5, either with stimulating B cell targets, bearing OVA/I-A^d or D^d, respectively, or with stimulatory anti-receptor antibodies coupled to Sepharose beads. As shown in Table III, anti-LFA-1 inhibited very well the responses of these T cells to Ag/MHC presented by the B cell lymphoma, A20-2J. The antibody had no effect on the responses of these T cells to polyvalent anti-receptor antibody, however.

From these experiments we concluded, as before, that LFA-1 did not play an essential role in T cell activation for IL-2 production, but rather was involved in the interaction of T cells with certain target cells, which did not include L cells and their derivatives.

Our future experiments will test whether LFA-1 functions when normal mouse fibroblasts are used as APC for T cell hybridomas, instead of L cells. This is the case in man, since Collins et al. (15) have shown that human dermal fibroblasts, induced to express class II molecules by gamma interferon (IFN-γ), can be killed by class II–specific cytotoxic T cells, and that this killing is blocked by anti-LFA-1 antibodies. With this in mind, we have been unable to show that Ag presentation by L cell lines becomes sensitive to anti-LFA-1 antibodies if the cells are preincubated with IFN-γ (data not shown); it remains to be determined if this is a property of L cells only or if it also extends to normal mouse fibroblasts.

Summary

We show that the LFA-1 molecule on T cells does not play a role in the stimulation of T cell hybridomas by certain targets, namely antigen presented by L cell derivatives or polyvalent anti-receptor antibody. These results suggest that LFA-1 may act by binding to ligands that are not present on all cells. We hope this result will help us and others to establish the true role of LFA-1 in T cell responses.

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