Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1194–1198, February 1988
Immunology

Expression of the T-cell surface molecule CD2 and an epitope-loss CD2 mutant to define the role of lymphocyte function-associated antigen 3 (LFA-3) in T-cell activation

(surface antigen/T lymphocytes/monoclonal antibodies)

BARBARA E. BIERER*†‡, ANDREW PETERSON§¶, JAMES BARBOSA||, BRIAN SEED§¶, AND STEVEN J. BURAKOFF†**

*Hematology Division, Department of Medicine, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115; †Division of Pediatric Oncology, Dana–Farber Cancer Institute, 44 Binney Street, Boston, MA 02115; §Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114; ||Molecular Diagnostics, Inc., West Haven, CT 06516; and Departments of ‡Medicine, ¶Genetics, and **Pediatrics, Harvard Medical School, Boston, MA 02115

Communicated by Baruj Benacerraf, October 23, 1987

ABSTRACT **To define the role of the CD2–lymphocyte function-associated antigen 3 (LFA-3) interaction in T-cell activation, we have expressed a cDNA encoding the human CD2 molecule in a murine antigen-specific T-cell hybridoma. Expression of the CD2 molecule greatly enhances T-cell responsiveness to antigen; this enhancement is inhibited by anti-CD2 and anti-LFA-3 monoclonal antibodies (mAbs). CD2$^+$ hybridomas produce interleukin 2 in response to combinations of anti-CD2 mAbs 9.6 and 9-1 and, in the presence of mAb 9-1, to sheep erythrocytes or to the LFA-3 antigen. Furthermore, hybridomas expressing a mutant CD2 molecule that has lost mAb 9.6 binding do not exhibit the enhanced response to antigen or the ability to respond to LFA-3 plus mAb 9-1, but these hybridomas retain the ability to respond to combinations of anti-CD2 mAbs. The role of the CD2–LFA-3 interaction in T-cell activation and the potential for other physiologic ligands for CD2 are discussed.**

The T-cell erythrocyte receptor, or CD2 (T11, Leu 5, lymphocyte function-associated antigen 2) molecule, is a 50-kDa protein that mediates T-cell adhesion to sheep (1, 2) and human (3–6) erythrocytes. Certain monoclonal antibodies (mAbs) directed against CD2 inhibit T-cell rosette formation (7–10), as well as cytolytic T-cell function (11, 12) and T-cell proliferation (11–14). In contrast to the inhibition of T-cell function by some individual anti-CD2 mAbs, certain pairs of anti-CD2 antibodies can directly initiate T-cell activation (15–17). This pathway of T-cell activation may be accessory-cell independent; its relation to T-cell activation via the T-cell receptor (TCR)–CD3 complex remains to be determined.

Recent studies have indicated that a natural ligand for CD2 is a broadly distributed cell-surface protein, lymphocyte function-associated antigen 3 (LFA-3). Studies of mAb inhibition of antigen-independent conjugate formation (18) and of thymocyte binding to thymic epithelial cells (19) have suggested that CD2 interacts with LFA-3. The demonstration that purified LFA-3 binds to CD2 (20, 21) and that rosetting of human erythrocytes to CD2-transfected COS cells is blocked by anti-LFA-3 mAb (6) has confirmed that the CD2–LFA-3 interaction functions in cell–cell adhesion.

Although the CD2–LFA-3 interaction has been shown to mediate T-cell adhesion, its role in T-cell activation remains to be defined. We have expressed the cDNA encoding the human CD2 molecule in a murine antigen-specific T-cell hybridoma. CD2 expression augments the T-cell response to antigen, and this enhancement is inhibited by anti-CD2 and anti-LFA-3 mAbs. CD2 expression confers upon the T cell the ability to produce interleukin 2 (IL-2) to pairs of anti-CD2 mAbs such as mAbs 9.6 and 9-1, and, in the presence of the anti-CD2 mAb 9-1, to sheep erythrocytes (RBC) or to murine L cells expressing the LFA-3 molecule. T-cell hybridomas expressing a point mutant deficient in mAb 9.6 binding and erythrocyte rosetting do not exhibit the enhanced response to antigen nor the ability to respond to LFA-3 plus mAb 9-1, but these hybridomas retain the ability to produce IL-2 to pairs of anti-CD2 mAbs.

MATERIALS AND METHODS

Cell Culture. Cells were grown in RPMI 1640 medium (M. A. Bioproducts, Walkersville, MD) supplemented with 10% heat-inactivated fetal bovine serum (Hazelton Research Products, Denver, PA), penicillin at 100 units/ml (GIBCO), streptomycin at 100 μg/ml (GIBCO), 10 mM Hepes (M. A. Bioproducts), 2 mM glutamine (GIBCO), and 50 μM 2-mercaptoethanol (Eastman Organic Chemicals, Rochester, NY). Murine L cells and L cell transfectants were maintained in monolayer culture in Dulbecco's modified Eagle's medium (DMEM)-10 supplemented with 10% heat-inactivated fetal bovine serum, penicillin, streptomycin, and glutamine, and cells were harvested for assay using 0.03% EDTA in phosphate-buffered saline.

mAbs. Murine antibodies directed against CD2 have been previously described: TS2/18 (IgG1) and TS1/8 (IgG1) (22); 9.6 (IgG2a) (8), a gift of J. Hansen (University of Washington Medical Center, Seattle); 9-1 (IgG3) (23), a gift of S. Y. Yang and B. Dupont (Sloan–Kettering Institute, New York). Additional anti-CD2 mAbs were obtained through the Third International Workshop on Leukocyte Typing (24). The following mAbs were also used: TS2/9 (murine IgG1) specific for human LFA-3 (22); F23.1 (murine IgG, specific for Vβ8 variable region of the murine TCR) (25); M17/5 (rat IgG2b, specific for murine LFA-1) (26); 145-2C11 (hamster IgG, specific for murine CD3) (27), a gift of J. Bluestone (Chicago).

Construction of Cell Lines. The CD2 cDNA (6) and the Q51L mutant (28) were inserted into the retroviral vector MNC′ (A.P., unpublished work). The MNC′ vector is essentially the same as the MNS vector described (29), except that the simian virus 40 promoter has been replaced with the immediate early promoter from the human cytomegalovirus.

Abbreviations: FACS, fluorescence-activated cell sorter; HLA, human leukocyte antigen; IL-2, interleukin 2; LFA, lymphocyte function-associated antigen; MHC, major histocompatibility complex; RBC, erythrocytes; TCR, antigen-specific T-cell receptor.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Producer lines were constructed, and infections were done as described (29). Murine L cells expressing the LFA-3 surface antigen were generated by whole cell human DNA transfer and selection by cell sorting with TS2/9 mAb (B.E.B., J.B., S. Herrmann, and S.J.B., unpublished work).

T-Cell Hybridoma Production. The murine T-cell hybridoma 155.16 was produced by the fusion of the HAT-sensitive thymoma BW5147 cell line with spleen cells from C57BL/6 mice primed *in vivo* and boosted *in vitro* with the human Epstein–Barr virus-transformed cell line JY (29). Hybridomas were screened for antigen-specific IL-2 production and cloned by limiting dilution. The hybridoma 155.16 was infected with the retroviruses containing CD2 and Q51L CD2 molecules by incubating 5×10^5 cells in 12 ml of supernatant from a confluent 10-cm^2 plate of MNC′ CD2 DAMP and MNC′Q51L DAMP producer cells with Polybrene (10 $\mu g \cdot ml^{-1}$). Hybridoma cells were infected with the retrovirus containing the *neo* gene alone in an equivalent manner (29). After 48 hr, hybridomas were cloned by limiting dilution in selection medium containing G418 (2.0 $mg \cdot ml^{-1}$; GIBCO). Neomycin-resistant cell lines were screened for CD2 expression by indirect immunofluorescence.

Cell-Surface Immunofluorescence and Cytofluorographic Analysis. Approximately 5×10^5 cells were incubated on ice in the dark for 30 min with saturating concentrations of mAb. Cells were washed, incubated with 5 μl of fluorescein-conjugated goat $F(ab')_2$ anti-mouse IgG antibody (FITC-GAM; Tago, Burlingame, CA) on ice for 30 min, washed, and fixed in 1% paraformaldehyde in phosphate-buffered saline. Five to ten thousand cells were analyzed on an EPIC V Coulter Counter.

Stimulation of IL-2 Production by T-Cell Hybridomas. T-cell hybridomas, 5×10^4 cells per well, were incubated with antibodies or varying numbers of irradiated (100 cGy from a cesium source) stimulator cells, sheep RBC (Granite Diagnostics, Burlington, NC) or murine RBC cells, with or without mAb, in 0.6 ml of complete media in 48-well flat bottom plates (Costar, Cambridge, MA). Cells were incubated for 24 hr in 5% CO_2 in air. Culture supernatants were harvested, frozen, thawed, titrated by serial 2-fold dilutions, and assayed for IL-2 by their ability to support proliferation of an IL-2-dependent murine T-cell line, CTLL-20 (30). Proliferation was assessed by the incorporation of [^{3}H]thymidine in a 4-hr pulse after a 20-hr incubation. Results are expressed relative to the [^{3}H]thymidine incorporation of CTLL-20 cells cultured with a standard rat Con A supernatant in which half-maximal incorporation is defined as 100 units of IL-2$\cdot ml^{-1}$.

RESULTS

Expression of the Human CD2 Molecule in a Murine Antigen-Specific T-Cell Hybridoma. The human leukocyte antigen (HLA)-DR-specific murine T-cell hybridoma, 155.16, is specific for HLA-DR antigens in that it produces IL-2 after stimulation by the human Epstein–Barr virus-transformed B-cell line JY (29). Stimulation of IL-2 production may be inhibited by mAb directed against HLA-DR. The hybridoma 155.16 expresses the Vβ8 variable region of the T-cell receptor identified by mAb F23.1 (25). A murine CD2 cDNA probe does not hybridize with mRNA prepared from the parent hybridoma (W. Jones and S.J.B., unpublished data).

The murine T-cell hybridoma 155.16 was infected with a retrovirus containing a cDNA encoding the human CD2 molecule (6). Infected cells were initially selected by resistance to G418 and screened for CD2 expression by indirect immunofluorescence using the anti-CD2 mAb TS2/18. The response of the CD2$^+$ hybridomas was compared to that of a cell line infected with a retroviral vector containing only the *neo* gene, hybridoma 16.M/9.

Surface expression of CD2 was examined with a panel of anti-CD2 mAbs. Flow cytometric analysis of hybridoma 16.M/9 and a representative CD2$^+$ hybridoma is shown in Fig. 1. Hybridoma 16.CD2-18 expressed the CD2 epitopes defined by the mAbs TS2/18, 9.6 (Fig. 1), as well as by mAbs 7E10, MT110, and MT910 (data not shown). Consistent with previous observations of human peripheral blood T cells (17, 31), the level of expression of the 9-1 epitope varied, but generally this level was less than that of the 9.6 epitope (Fig. 1). Expression of the murine TCR defined by mAb F23.1 was equivalent on hybridoma 16.M/9 and 16.CD2-18 (Fig. 1), as was the expression of LFA-1 (data not shown). The CD2$^+$ hybridomas 16.CD2-15 and 16.CD2-43 showed equivalent surface expression of these antigens (data not shown).

The Effect of CD2 Expression on Antigen-Specific T-Cell Stimulation. In comparison to hybridoma 16.M/9, the hybridomas expressing CD2 demonstrated a 4- to 50-fold increase in the IL-2 response to stimulation by the JY cell line (Fig. 2*A*). Differences in responsiveness to JY stimula-

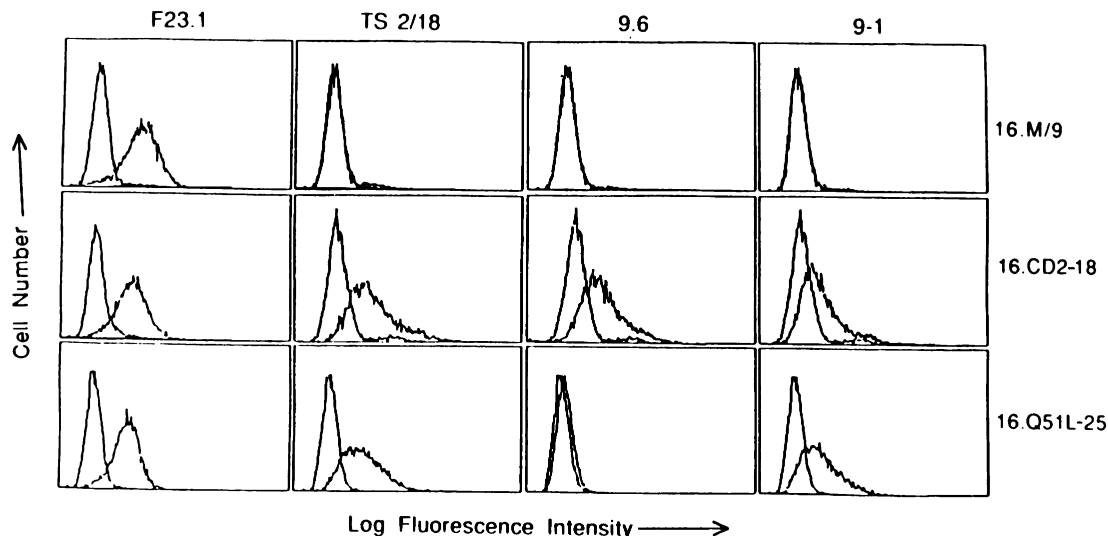

FIG. 1. Expression of the murine TCR (F23.1) and human CD2 (epitopes TS2/18, 9.6, and 9-1) molecules of murine T-cell hybridomas. Representative flow cytometric histograms are shown for hybridomas infected with retroviral vector encoding the neomycin resistant gene alone (16.M/9, *Upper*), the native CD2 cDNA (16.CD2-18, *Middle*), or the mutant Q51L CD2 cDNA (16.Q51L-25, *Lower*).

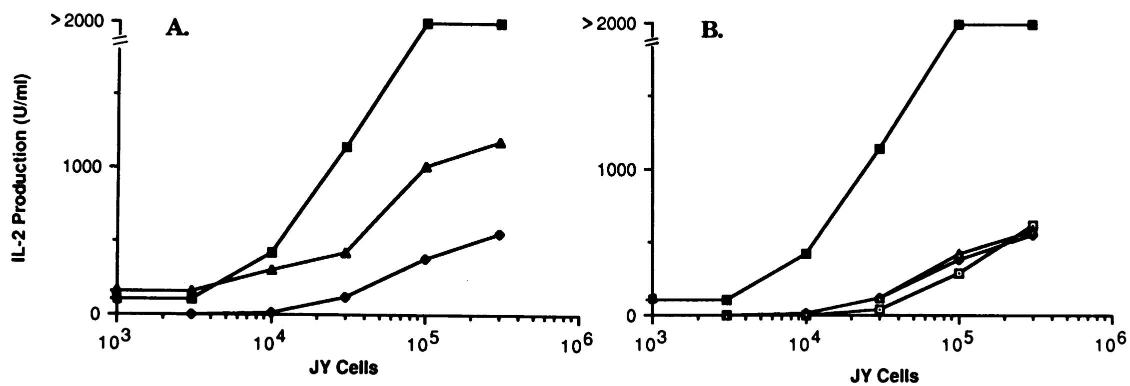

FIG. 2. Antigen-specific stimulation of IL-2 production by hybridomas expressing either the CD2 (*A*) or Q51L CD2 (*B*) molecules. T-cell hybridomas (5×10^4) were cocultured with increasing numbers of irradiated JY cells, as indicated. Hybridomas: ◆, 16.M/9; ▲, 16.CD2-18; ■, 16.CD2-43; △, 16.Q51L-9; ⊡, 16.Q51L-25.

tion by CD2$^+$ hybridomas reflected clonal variation of the cell lines and was not explained by differences in CD2 or TCR–CD3 surface expression. Antigen-specific stimulation of the parent hybridoma 155.16, hybridoma 16.M/9, and the CD2$^+$ hybridomas was inhibited by a mAb to LFA-1 expressed on the hybridoma (Table 1). However, the response of the CD2$^+$ hybridomas, but not that of the parent hybridoma or 16.M/9, was inhibited by incubation with the anti-CD2 mAbs TS2/18, TS1/8, and 9.6, and with the anti-LFA-3 mAb, TS2/9 (Table 1). This suggested that CD2 expressed on the hybridoma interacts with LFA-3 on the JY stimulator cells and that in the absence of CD2 expression, LFA-3 on the stimulator cell does not participate in the activation of the hybridoma.

Stimulation of IL-2 Production by Pairs of Anti-CD2 mAbs, by Sheep RBC Plus mAb 9-1, or by LFA-3 Plus mAb 9-1. Certain pairwise combinations of anti-CD2 mAbs have been shown to stimulate T-cell proliferation and effector function (15–17, 31). Incubation of the CD2$^+$ hybridomas with a number of anti-CD2 mAbs, such as 9.6 and 7E10 each in combination with mAb 9-1, stimulated IL-2 production by these CD2$^+$ hybridomas (Table 2). Thus, antibodies directed against the expressed CD2 molecule are able to trigger activation of the murine hybridoma.

Human T cells are known to form rosettes with sheep or human erythrocytes, but not with mouse erythrocytes (5, 32). The CD2 molecule has been shown to interact with LFA-3 on the human erythrocyte (4, 6) or with the sheep homologue of LFA-3 (33). The murine hybridomas expressing the human CD2 molecule were found to produce IL-2 in response to stimulation with sheep RBC in the presence of the anti-CD2 mAb 9-1 (Table 3, Exp. 1). In contrast, no IL-2 was produced when the CD2$^+$ hybridomas were cultured with sheep RBC alone or with murine RBC in the presence of mAb 9-1 (Table 3, Exp. 1).

To evaluate the functional consequences of the interaction of CD2 with the LFA-3 molecule, murine L cells were transfected with human genomic DNA and sorted for LFA-3 surface expression by indirect immunofluorescence. Hybridomas expressing the native CD2 molecule did not produce IL-2 in response to stimulation with mAb 9-1 alone, LFA-3$^+$ L cells alone, or to the parent L cell plus mAb 9-1 (Table 3, Exp. 2). However, stimulation of the CD2$^+$ hybridomas with LFA-3$^+$ L cells in the presence of mAb 9-1 (Table 3, Exp. 2), but not mAb 9.6 (data not shown), induced IL-2 production. Thus, LFA-3 binding to CD2 provides one signal for T-cell activation. LFA-3 binding appears to replace the signal produced by binding mAb 9.6 or by the LFA-3 homologue expressed on sheep erythrocytes.

A 9.6-Epitope-Loss Mutant of CD2 Can Respond to Pairs of Anti-CD2 mAbs, but This Mutant Cannot Respond to LFA-3. The generation of mutants of the CD2 molecule that have specifically lost anti-CD2 mAb reactivity has been described (28). The epitope-loss CD2 mutant chosen for the present study was selected for loss of binding of mAb 9.6. The mutant CD2 protein could bind other anti-CD2 mAbs that bind to the same region of CD2 but does not form rosettes with human erythrocytes (28). This CD2 mutant, called Q51L, has a single nucleotide change leading to a leucine-for-glutamine substitution at position 51.

The cDNA encoding the Q51L CD2 mutant was introduced into a retroviral vector, which was then used to infect the murine hybridoma 155.16. Hybridomas were selected for resistance to G418 and screened by indirect immunofluorescence for expression of TS2/18, a CD2 epitope, the expression of which was unaffected by the mutation. Three

Table 1. Stimulation of CD2$^+$ hybridomas by JY cells is inhibited by anti-CD2 and anti-LFA-3 mAb

JY	mAb	Antigen recognized	IL-2 production, units·ml^{-1}			
			155.16	16.M/9	16.CD2-18	16.CD2-43
–			<10	<10	<10	<10
+	None	None	192	90	1373	1042
+	M17/5	LFA-1	25	23	202	131
+	TS2/18	CD2	190	86	146	72
+	TS1/8	CD2	221	123	174	79
+	9.6	CD2	152	118	80	51
+	TS2/9	LFA-3	169	94	106	44

Hybridoma cells (5×10^4) were cultured with 10^5 irradiated JY cells, with or without mAb, for 24 hr. Supernatants were harvested and assayed for the presence of IL-2 as described. Data are presented as units of IL-2 activity per ml^{-1} relative to the incorporation of radioactivity after stimulation with a standard rat Con A supernatant. mAbs M17/5 and TS1/8 were used at a 1:20 dilution of culture supernatant, mAbs TS2/18 and TS2/9 as a 1:1000 dilution of ascites fluid, and mAb 9.6 at 1 μg·ml^{-1}. The experiment is representative of four separate experiments.

Table 2. Stimulation of IL-2 production by pairs of anti-CD2 mAbs

	IL-2 production, units·ml^{-1}		
mAb added	16.M/9	16.CD2-18	16.CD2-43
0	<10	<10	<10
9.6	<10	<10	<10
7E10	<10	<10	<10
9-1	<10	<10	<10
9.6 + 9-1	<10	1226	1301
7E10 + 9-1	<10	926	637

Hybridomas were cultured, and supernatants were harvested as described in the legend for Table 1. mAbs 9.6, 7E10, and 9-1 were used at a final concentration of 1 μg·ml^{-1}. The results shown are representative of three separate experiments.

hybridomas expressing the Q51L CD2 molecule were chosen for study because the surface density of the mutant CD2 molecule was equivalent to the native CD2$^+$ counterpart (Fig. 1). The Q51L CD2 molecule expressed in hybridoma 155.16 did not react with mAb 9.6 (Fig. 1), had diminished expression of the 7E10 epitope, but had retained binding of mAbs MT110 and MT910 (data not shown) and of mAb 9-1 (Fig. 1). The Q51L CD2$^+$ hybridomas expressed the murine TCR (Fig. 1) and the LFA-1 molecule (data not shown) at levels equivalent to the parental hybridoma and to hybridomas that expressed the wild-type CD2.

The hybridomas expressing the Q51L CD2 molecule showed no greater response to stimulation with JY cells than did hybridoma 16.M/9 (Fig. 2*B*). Coculture of the Q51L CD2$^+$ hybridomas with sheep RBC in the presence of mAb 9-1 did not induce IL-2 production (Table 4, Exp. 1). Furthermore, neither the non-CD2-expressing cell line 16.M/9 nor hybridomas expressing the Q51L CD2 molecule were stimulated to produce IL-2 by LFA-3$^+$ L cells plus mAb 9-1 (Table 4, Exp. 2), confirming the observation that the mutant CD2 molecule was unable to interact with LFA-3.

The Q51L CD2$^+$ hybridomas, however, could produce equivalent amounts of IL-2 as the CD2$^+$ hybridomas upon stimulation of the TCR–CD3 complex with the anti-TCR mAb F23.1 attached to solid support or by incubation of the hybridomas with the calcium ionophore ionomycin and phorbol 12-myristate 13-acetate (PMA) (Table 4, Exp. 3). Hybridomas expressing the mutant Q51L CD2 molecule did not produce IL-2 upon stimulation with the combination of mAbs 9.6 and 9-1 nor with mAbs 7E10 and 9-1 (Table 4, Exp. 4). However, these hybridomas did produce IL-2, like the wild-type CD2$^+$ hybridomas, upon stimulation with either mAbs MT110 or MT910 in the presence of mAb 9-1. Therefore, the Q51L CD2 molecule is defective, not in T-cell activation but in the ability to use LFA-3 or mAb 9.6 for activation.

Table 3. Stimulation of IL-2 production by LFA-3 plus mAb 9-1

	IL-2 production, units·ml^{-1}		
Stimulation	16.M/9	16.CD2-15	16.CD2-18
Exp. 1			
9-1	<10	18	14
Murine RBC	<10	<10	<10
Murine RBC + mAb 9-1	<10	<10	<10
Sheep RBC	<10	<10	<10
Sheep RBC + mAb 9-1	<10	2035	2162
Exp. 2			
9-1	<10	<10	<10
L cells	<10	<10	<10
LFA-3$^+$ L cells	<10	<10	<10
L cells + mAb 9-1	<10	<10	<10
LFA-3$^+$ L cells + mAb 9-1	<10	236	146

Hybridomas were cultured, and supernatants were harvested as described in the legend for Table 1. mAb 9-1 was used at a final concentration of 1 μg·ml^{-1}. The RBC/hybridoma ratio was 1000:1. Irradiated (150 cGy) L cells were used at 2 × 10^5 cells per well. Each experiment is representative of three separate experiments.

DISCUSSION

In this report, we have shown that the human CD2 surface molecule may be expressed in a murine T-cell hybridoma and that the molecule is functional. CD2 expression enhances stimulation of the hybridoma by antigen, and this enhancement is inhibited by anti-CD2 and anti-LFA-3 mAbs, consistent with the proposal that CD2 and LFA-3 are involved in a common pathway of antigen-independent conjugate formation (18). We show that the CD2$^+$ T-cell hybridoma may be stimulated either (*i*) with appropriate pairs of

Table 4. Stimulation of IL-2 production by CD2$^+$ and Q51L CD2$^+$ hybridomas

	IL-2 production, units·ml^{-1}				
Stimulation	16.M/9	16.CD2-18	16.Q51L-9	16.Q51L-25	16.Q51L-27
Exp. 1					
Sheep RBC	<10	<10	<10	33	<10
Sheep RBC + mAb 9-1	<10	947	<10	30	<10
Exp. 2					
mAb 9-1	<10	<10	<10	<10	ND
LFA-3$^+$ L cells	<10	<10	<10	<10	ND
LFA-3$^+$ L cells + mAb 9-1	<10	446	<10	<10	ND
Exp. 3					
mAb F23.1 (0.3%)	525	976	1080	1003	ND
Ionomycin + PMA	103	401	269	369	ND
Exp. 4					
0	<10	<10	<10	<10	<10
mAbs 7E10 + 9-1	<10	306	<10	<10	<10
mAbs 9.6 + 9-1	<10	292	<10	<10	<10
mAbs MT110 + 9-1	<10	547	260	253	258
mAbs MT910 + 9-1	<10	568	277	159	275

Hybridomas were cultured, and supernatants were harvested as described in the legend to Table 1. mAbs were used at a final concentration of 1 μg·ml^{-1}. Incubation of the hybridomas with each anti-CD2 mAb alone did not stimulate IL-2 production. F23.1 was attached to solid support by incubating culture supernatant in 48-well plates overnight in the cold on a rocker platform. Wells were washed three times with PBS, and hybridomas were added. Ionomycin (0.2 μM) and phorbol 12-myristate 13-acetate (PMA; 5 ng·ml^{-1}) were added at the start of the assay. ND, not done.

anti-CD2 mAbs, (*ii*) by sheep RBC but not murine RBC in the presence of mAb 9-1, or (*iii*) by murine L cells transfected with LFA-3 in the presence of mAb 9-1. This demonstrates that the CD2–LFA-3 interaction can mediate antigen-independent T-cell activation and may be involved in antigen-dependent activation. The data pressented here provide evidence that the interaction of the CD2 molecule with its naturally occurring ligand, LFA-3, may induce one signal for T-cell activation.

The murine T-cell hybridomas expressing the human CD2 molecule demonstrate a 4- to 50-fold enhanced response to antigen stimulation by JY cells, as compared to either the parent cell line or a hybridoma infected with the *neo* gene alone, 16.M/9. The ability of anti-CD2 and anti-LFA-3 mAbs to inhibit the stimulation of the $CD2^+$ hybridomas suggests that CD2 on the hybridoma is interacting with LFA-3 on the stimulator cell. That the CD2–LFA-3 interaction functions in antigen-specific stimulation is further supported by the observation that a mutant CD2 molecule (Q51L) that does not bind mAb 9.6, when expressed in the hybridoma, fails to augment the antigen-specific response of the hybridoma. The Q51L $CD2^+$ hybridomas are unable to produce IL-2 in response to either sheep RBC or $LFA\text{-}3^+$ L cells in the presence of mAb 9-1, but these hybridomas are able to respond to pairs of anti-CD2 mAbs (MT110 plus 9-1; MT910 plus 9-1). Therefore, the CD2 molecule can function independently of LFA-3 binding, but the CD2 epitope defined by mAb 9.6 is required for CD2 to function in antigen-dependent activation of the T cell.

Both the $CD2^+$ and Q51L $CD2^+$ hybridomas produce IL-2 in response to mAb 9-1 plus anti-CD3 mAb (data not shown). This demonstrates that binding of mAb 9-1 is sufficient to allow the CD2 pathway to synergize with the activation signal mediated by the TCR–CD3 antigen-specific pathway (17, 31). These results, together with the observation that the antigen-specific response of the Q51L $CD2^+$ hybridoma is equivalent to hybridoma 16.M/9, suggest that the natural ligand for the 9-1 CD2 epitope, if one exists, is not expressed on the human Epstein–Barr virus-transformed cell line JY, or, in the physiologic state, depends on the ability of CD2 to bind LFA-3. The natural ligand may be a soluble factor or lymphokine that requires prior LFA-3 binding for release or for binding. It is also possible that the ligand complementary to mAb 9-1 is an inhibitor molecule on the hybridoma, which is lost upon binding of the T cell with either LFA-3, the appropriate stimulator cell, or anti-CD2 mAb.

The results presented here demonstrate that the interaction of CD2 with its naturally occurring ligand LFA-3 plays a role in T-cell activation. The CD2–LFA-3 interaction is not sufficient for T-cell stimulation, but requires the presence of a second signal. Others have observed that complementary signals are required for the activation through the CD2 pathway: pairs of anti-CD2 mAbs directed at discrete CD2 determinants (15–17), anti-CD2 mAb in the presence of activators of protein kinase C (34), sheep RBC in the presence of anti-T11.3 mAb (33), or anti-CD2 mAb in the presence of an anti-CD3 mAb (17, 31). We have shown that the CD2–LFA-3 interaction functions physiologically upon interaction of the T cell with its stimulator cell to enhance T-cell responsiveness and have localized one region on the CD2 molecule critical for functional interaction with LFA-3.

This work was supported by National Institutes of Health Grant CA 34129 (S.J.B.) and by a grant from Hoechst AG (A.P. and B.S.). B.E.B. is a recipient of a Clinician-Scientist Award from the American Heart Association.

1. Bach, J. F., Dormont, J., Dardenne, M. & Balner, H. (1969) *Transplantation* **8,** 265–280.
2. Brain, P., Gordon, J. & Willets, W. A. (1970) *Clin. Exp. Immunol.* **6,** 681–688.
3. Baxley, G., Bishop, G. B., Cooper, A. G. & Wortis, H. H. (1973) *Clin. Exp. Immunol.* **15,** 385–392.
4. Plunkett, M. L., Sanders, M. E., Selvaraj, P., Dustin, M. L. & Springer, T. A. (1987) *J. Exp. Med.* **165,** 664–676.
5. Nalet, V. & Fournier, C. (1985) *Cell. Immunol.* **96,** 126–136.
6. Seed, B. & Aruffo, A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3365–3369.
7. Howard, F. D., Ledbetter, J. A., Wong, J., Bieber, C. P., Stinson, E. B. & Herzenberg, L. A. (1981) *J. Immunol.* **126,** 2117–2122.
8. Kamoun, M., Martin, P. J., Hansen, J. A., Brown, M. A., Siadak, A. W. & Nowinski, R. C. (1981) *J. Exp. Med.* **153,** 207–212.
9. Verbi, W., Greaves, M. F., Schneider, C., Koubek, K., Janossy, G., Stein, H., Kung, P. & Goldstein, G. (1982) *Eur. J. Immunol.* **12,** 81–86.
10. Bernard, A., Gelin, C., Raynal, B., Pham, D., Gosses, C. & Boumsell, A. (1982) *J. Exp. Med.* **155,** 1317–1333.
11. Van Wauwe, J., Goossens, J., Decock, W., Kung, P. & Goldstein, G. (1981) *Immunology* **44,** 865–871.
12. Martin, P. J., Longston, G., Ledbetter, J. A., Newman, W., Braun, M. P., Beatty, P. G. & Hansen, J. A. (1983) *J. Immunol.* **131,** 180–185.
13. Krensky, A. M., Sanchez-Madrid, F., Robbins, E., Nagy, J. A., Springer, T. A. & Burakoff, S. J. (1983) *J. Immunol.* **131,** 611–616.
14. Palacios, R. & Martinez-Maza, O. (1982) *J. Immunol.* **129,** 2479–2485.
15. Meuer, S. C., Hussey, R. E., Fabbi, M., Fox, D., Acuto, O., Fitzgerald, K. A., Hodgdon, J. C., Protentis, J. P., Schlossman, S. F. & Reinherz, E. L. (1984) *Cell* **36,** 897–906.
16. Brottier, P., Boumsell, L., Gelin, C. & Bernard, A. (1985) *J. Immunol.* **135,** 1624–1631.
17. Yang, S. Y., Chouaib, S. & Dupont, B. (1986) *J. Immunol.* **137,** 1097–1100.
18. Shaw, S., Luce, G. E. G., Quinones, R., Gress, R. E., Springer, T. A. & Sanders, M. E. (1986) *Nature (London)* **323,** 262–264.
19. Vollger, L. W., Tuck, D. T., Springer, T. A., Haynes, B. F. & Singer, K. H. (1987) *J. Immunol.* **138,** 358–363.
20. Dustin, M. L., Sanders, M. E., Shaw, S. & Springer, T. A. (1987) *J. Exp. Med.* **165,** 677–692.
21. Takai, Y., Reed, M., Burakoff, S. J. & Herrmann, S. H. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6864–6868.
22. Sanchez-Madrid, F., Krensky, A. M., Ware, C. F., Robbins, E., Strominger, J. L., Burakoff, S. J. & Springer, T. A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7489–7493.
23. Bernard, A., Brottier, P., Georget, E., Lepage, V. & Boumsell, L. (1986) in *Leukocyte Typing II*, eds. Reinherz, E. L., Haynes, B. F., Nadler, L. M. & Bernstein, I. D. (Springer, New York), pp. 53–66.
24. McMichael, A. J., ed. (1987) *Leukocyte Typing III* (Oxford Univ. Press, Oxford).
25. Staerz, U., Rammensee, H. G., Benedetto, J. D. & Bevan, M. J. (1985) *J. Immunol.* **134,** 3994–4000.
26. Springer, T. A., Davignon, D., Ho, M.-K., Kurzinger, K., Martz, E. & Sanchez-Madrid, F. (1982) *Immunol. Rev.* **68,** 171–198.
27. Leo, C., Foo, M., Sachs, D. H., Samelson, L. E. & Bluestone, J. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 1374–1378.
28. Peterson, A. & Seed, B. (1987) *Nature (London)* **329,** 842–846.
29. Sleckman, B. P., Peterson, A., Jones, W. K., Foran, J. A., Greenstein, J. L., Seed, B. & Burakoff, S. J. (1987) *Nature (London)* **328,** 351–353.
30. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120,** 2027–2032.
31. Yang, S. Y., Rhee, S., Angelos, G. & Dupont, B. (1987) in *Leukocyte Typing III*, ed. McMichael, A. J. (Oxford Univ. Press, Oxford), pp. 113–116.
32. Johansen, K. S., Johansen, S. T. & Talmage, D. W. (1974) *J. Allergy Clin. Immunol.* **54,** 86–94.
33. Hunig, T., Tiefenthaler, G., Meyer zum Buschenfelde, K.-H. & Meuer, S. C. (1987) *Nature (London)* **326,** 298–301.
34. Holter, W., Fischer, G. F., Majdic, O., Stockinger, H. & Knapp, W. (1986) *J. Exp. Med.* **163,** 654–664.