

SYNERGISTIC T CELL ACTIVATION VIA THE PHYSIOLOGICAL LIGANDS FOR CD2 AND THE T CELL RECEPTOR

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T cells may be activated by the antigen-specific T cell receptor (TCR)-CD3 complex interacting either with foreign antigen in association with self-major histocompatibility complex (MHC) proteins or by foreign MHC proteins. T cells may also be activated by the cell surface receptor CD2 (T11, LFA-2, the T cell erythrocyte receptor), a molecule of M_r 50,000 expressed on all human thymocytes and T cells. Pairs of mAbs directed against different epitopes on CD2 will stimulate T cells (1-3). While both the antigen-specific TCR and the CD2 pathways of T cell activation may be triggered independently, several lines of evidence suggest that they are functionally interrelated. Incubation of resting T cells with a nonstimulatory anti-CD3 mAb in the presence of an anti-CD2 mAb directed against a specific epitope (9-1 mAb) induces T cell stimulation (3). Modulation of the TCR-CD3 complex by incubation with an anti-CD3 mAb prevents subsequent activation by stimulatory pairs of anti-CD2 mAb (1, 2, 4-6). Whether CD3 expression is required for CD2-dependent activation or whether CD3 modulation induces a state of refractoriness to subsequent stimulation is not clear. Some (7), but not all (6), CD3-deficient mutant T cell lines may be stimulated by pairs of anti-CD2 mAb. On the other hand, while modulation of surface CD2 expression does not inhibit anti-CD3-induced calcium mobilization (5), incubation of T cells with certain anti-CD2 mAb inhibits T cell stimulation through the TCR-CD3 complex (8-11), suggesting that CD2 may regulate T cell responses.

A natural ligand for CD2 has been found to be lymphocyte function-associated antigen 3 (LFA-3),¹ a widely distributed cell surface glycoprotein of M_r

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¹ *Abbreviations used in this paper:* CD, cluster of differentiation; GAM, goat F(ab)₂ anti-mouse IgG; LFA, lymphocyte function-associated.

55,000–70,000. CD2 on the T cell participates in antigen-independent adhesion or conjugate formation by binding to LFA-3 on the stimulator or target cell (12–15). In addition to T cell adhesion, the CD2/LFA-3 interaction plays a role in T cell activation (16–18). Incubation of resting T cells with sheep erythrocytes, which bear an LFA-3 homologue, in the presence of one anti-CD2 mAb induces human T cell proliferation (16). Murine L cells transfected with and expressing human LFA-3 together with an anti-CD3 mAb or with suboptimal concentrations of phytohemagglutinin stimulate proliferation of human peripheral blood T cells (17).

To investigate the interaction of the CD2 activation pathway with the antigen-specific TCR pathway, we have expressed a cDNA encoding the human CD2 molecule in a murine T cell hybridoma that responds to HLA-DR antigens. We have previously shown that this hybridoma produces IL-2 upon stimulation with pairs of anti-CD2 mAb, such as 9.6 and 9-1 mAb, or with 9-1 mAb in the presence of murine L cells expressing human LFA-3 (18), demonstrating that the CD2 pathway of activation is functional. CD2 expression markedly enhances IL-2 production in response to LFA-3⁺ antigen-bearing stimulator cells, and this stimulation is inhibited by anti-CD2 and anti-LFA-3 mAb.

To further define the role of LFA-3 in antigen-dependent T cell activation, we have studied the ability of the purified ligands of CD2 and the TCR to stimulate the hybridoma. The HLA-DR-specific murine T cell hybridoma expressing CD2 produced IL-2 after stimulation with liposomes containing purified LFA-3 and HLA-DR, the physiological ligands for CD2 and the TCR, respectively, suggesting that complementary interactions between the TCR-CD3 complex and the CD2 pathway may regulate lymphocyte activation. We have also constructed a cytoplasmic deletion mutant of CD2, CD2 Δ B, to examine the role of the CD2/LFA-3 interaction. The behavior of hybridomas expressing CD2 Δ B allow a clear distinction to be made between the roles of adhesion and that of activation for the CD2/LFA-3 interaction. Deletion of the cytoplasmic domain of CD2 did not alter binding of LFA-3 but eliminated the ability of CD2 to increase the response of the hybridoma to liposomes containing both HLA-DR and LFA-3. These results suggest that the CD2/LFA-3 interaction not only plays a role in cell-cell adhesion but provides a stimulatory signal for T cell activation.

Materials and Methods

Cell Culture. Cell lines were grown in DMEM (M. A. Bioproducts, Bethesda, MD) supplemented with 10% heat-inactivated FCS (Hazelton Research Products, Denver, PA), penicillin 100 U/ml (Gibco Laboratories, Grand Island, New York), streptomycin 100 μ g/ml (Gibco Laboratories), 10 mM HEPES (M. A. Bioproducts), and 2 mM glutamine (Gibco Laboratories). All assays for IL-2 were performed in RPMI-1640 (M. A. Bioproducts) supplemented as above and including 50 μ M 2-ME (Eastman Organic Chemicals, Rochester, NY).

Monoclonal Antibodies. Antibodies against LFA-3 (TS2/9) and CD2 (TS2/18) have been previously described (19), and were used as ascites fluid or purified antibody. Additional anti-CD2 mAb were obtained through the Third International Workshop on Leukocyte Typing (20). F23.1, murine IgG, specific for V β 8 variable region of the murine TCR (21); 100.30.3 rat IgG2b, specific for murine H-2k^k (22); and LB3.1, murine IgG1, specific for monomorphic determinants of HLA-DR (23), were also used.

Production of CD2- and CD2 Δ B-expressing Cell Lines. The CD2-expressing T cell hybridomas were constructed as previously described (18). CD2 Δ B was constructed by cleavage of a CD2 cDNA with Ban II, treatment of the end with T4 DNA polymerase followed by ligation

tion to 12-mer Nhe I linkers (stop codon linkers; New England Biolabs, Beverly, MA). Digestion of the resulting plasmid with Hind III and Bam HI released an 800-bp fragment; this was then introduced into the retroviral vector MNCstuffer (A. Peterson, unpublished). This deletion removes the COOH-terminal 100 amino acids of CD2 and replaces them with a serine.

CD2 and CD2 Δ B producer lines were constructed, and infections were done as described (24). Briefly, the murine T cell hybridoma 155.16, produced by the fusion of the HAT-sensitive thymoma BW5147 with spleen cells from C57BL/6 mice primed with the human EBV-transformed cell line JY (24), was infected with defective retroviruses that impart resistance to G418 and either CD2 or CD2 Δ B expression to the host cell. Neomycin-resistant cell lines were screened for wild-type or mutant CD2 expression by indirect immunofluorescence.

Immunofluorescent Flow Cytometry. 5×10^5 hybridoma cells were washed twice with PBS containing 2% FCS and 0.02% sodium azide, and then incubated at 4°C in the dark for 30 min with saturating concentrations of mAb. Cells were washed as above and incubated with 5 μ l FITC-conjugated goat F(ab')₂ anti-mouse IgG antibody (FITC-GAM) (Tago Inc., Burlingame, CA) at 4°C for 30 min. Cells were washed twice and incubated with 10 nM propidium iodide (Sigma Chemical Co., St. Louis, MO) in PBS. 5 – 10×10^3 cells were analyzed on an Epic V (Coulter Electronics, Hialeah, FL) fluorescence-activated cell sorter, during which dead cells were excluded by propidium iodide fluorescence.

Purification of LFA-3. The purification of LFA-3 by affinity chromatography has been described (16). In brief, purified anti-LFA-3 mAb TS 2/9 was coupled to cyanogen bromide-activated Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ) as described (16) to prepare the anti-LFA-3 affinity columns. JY cells were solubilized with 2% Triton X-100 in PBS and were filtered. LFA-3 was purified from the lysate using an anti-LFA-3 column, washed with 0.5% Triton X-100 in PBS, and eluted as described (16). The eluted protein was homogeneous as shown by SDS-PAGE and retained serological activity as demonstrated by dot blot analysis.

Preparation of HLA-DR and LFA-3 Liposomes. For preparation of HLA-DR, JY membranes were solubilized with 4% Nonidet P-40 as described (26). The HLA-DR antigen was immunoaffinity purified by passage of solubilized membranes through preclearing columns of Sepharose CL-4B, normal rabbit serum coupled to Sepharose CL-4B, and protein A-Sepharose, followed by an anti-HLA-DR column made by coupling the mAb LB3.1 (23) to protein A-Sepharose. The column was eluted with 50 mM glycine/0.1% sodium deoxycholate, pH 11.5. The fractions were neutralized with 2 M glycine, pH 2, dialyzed and concentrated by ultrafiltration. The purified, detergent-solubilized HLA-DR (40 μ g), purified LFA-3 (20 μ g), and purified HLA-DR (40 μ g) with LFA-3 (20 μ g) (DR + LFA-3) were each combined with detergent-solubilized JY membrane lipids (800 μ g) from a chloroform/methanol extraction. Liposomes were formed by removal of the detergent by dialysis, as described previously (23). The resulting liposomes were pelleted by centrifugation at 175,000 *g* for 1 h and resuspended in Tris-buffered saline.

Stimulation of IL-2 Production by T Cell Hybridomas. 5×10^4 T cell hybridomas were incubated with irradiated (100 cGy from a cesium source) JY stimulator cells or liposomes as indicated, with or without mAb, in 0.6 ml RPMI-1640 complete media in 48-well flat-bottom plates (Costar, Cambridge, MA). Cells were incubated for 24 h at 37°C in 5% CO₂ in air. Culture supernatants were harvested, frozen, thawed, titrated by serial twofold dilutions, and assayed for the presence of IL-2 by their ability to support the proliferation of an IL-2 dependent murine T cell line, CTLL-20 (27). Proliferation was assessed by the incorporation of [³H]thymidine in a 4-h pulse after a 20-h incubation. Results are expressed relative to the [³H]thymidine incorporation of CTLL-20 cultured with a standard rat Con A supernatant, in which half-maximal incorporation is defined as 100 U IL-2/ml.

Results

T Cell Hybridomas Expressing CD2 Respond to Antigen Stimulation and to Liposomes Containing HLA-DR and LFA-3. A murine T cell hybridoma, 155.16, was produced by the fusion of the murine AKR thymoma BW5147 with C57BL/6 spleen cells stimulated with the human EBV-transformed JY cell line and screened for antigen-specific

IL-2 production. The parent cell line 155.16, which produced IL-2 in response to stimulation with HLA-DR antigens (24), was infected with a defective retrovirus that imparts resistance to G418 and surface expression of the human CD2 molecule as described (18). We have previously demonstrated that murine T cell hybridomas expressing CD2 responded to pairs of anti-CD2 mAb such as 9.6 plus 9-1 mAb, or to the combination of 9-1 mAb and either sheep erythrocytes or murine L cells expressing human LFA-3 (18). This suggests that CD2 expressed by the murine hybridoma was functional and that LFA-3 may replace the signal provided by 9.6 mAb binding.

Expression of the CD2 molecule in the murine hybridoma 155.16 markedly enhanced IL-2 production in comparison to the parent hybridoma in response to antigen-specific stimulation with the HLA-DR⁺ JY cell line (Fig. 1). While there was clonal variability in responsiveness, the CD2⁺ hybridomas produced an average of 10-fold (range 3-30-fold) more IL-2 than the parent cell line to the same number of JY stimulator cells (data not shown). CD2⁺ hybridomas that express equivalent levels of CD2 and the TCR, as assessed by indirect immunofluorescence, were compared (data not shown). In response to JY cell stimulation, 16.CD2-15 produced approximately 10-20-fold more IL-2, and 16.CD2-43 produced three- to sixfold more IL-2, compared with the parent line. Stimulation of the CD2⁺ hybridomas, but not the parent, was inhibited by mAb directed against either CD2, expressed on the hybridoma, or LFA-3, expressed on the JY stimulator cells, suggesting that the CD2/LFA-3 interaction augmented the antigen-dependent response (Fig. 1).

To define the role of LFA-3 in antigen-dependent T cell stimulation, we investigated whether purified LFA-3 and HLA-DR proteins incorporated into liposomes would stimulate the CD2⁺ murine T cell hybridomas. LFA-3 and HLA-DR antigens were immunoaffinity purified from JY cells and the purified proteins were incorporated into liposomes. Neither the parent nor the CD2⁺ hybridomas produced IL-2 when liposomes containing only purified HLA-DR antigens were added (Table I). However, the CD2⁺ hybridomas, 16.CD2-15 and 16.CD2-18, but not the parent hybridoma, produced IL-2 in response to incubation with liposomes containing both LFA-3 and HLA-DR antigens incorporated into the same liposome (DR + LFA-3-liposomes) (Table I). LFA-3 liposomes alone did not stimulate IL-2 production. Purified HLA-A2 incorporated into liposomes with HLA-DR antigens

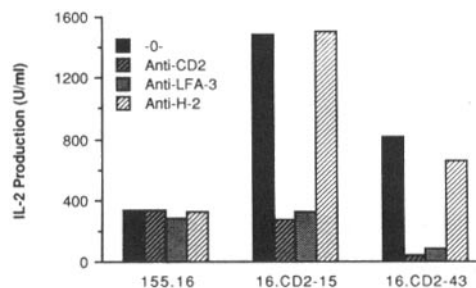


FIGURE 1. Inhibition of antigen-specific stimulation of IL-2 production by the murine T cell hybridoma 155.16 and the CD2⁺ hybridomas 16.CD2-15 and 16.CD2-43. 5×10^4 hybridoma cells were cultured with irradiated (100 cGy from a cesium source) JY stimulator cells, as described in Materials and Methods, in the presence or absence of mAb. The anti-CD2 mAb TS2/18 and anti-LFA-3 mAb TS2/9 were used at a 1:1,200 dilution of ascites fluid. The anti-H2k^k mAb 100.30.3 was used at a 10% final concentration of culture supernatant. All mAb concentrations were saturating. Data are presented as units of IL-2 activity per milliliter described in Materials and Methods.

TABLE I
HLA-DR + LFA-3-containing Liposomes Stimulate IL-2 Production by CD2⁺ Hybridomas

Stimulant	IL-2 production		
	155.16	16.CD2-15	16.CD2-18
	<i>U/ml</i>		
None	<10	<10	<10
JY cells	165	1,660	445
HLA-DR liposomes	<10	<10	<10
LFA-3 liposomes	<10	<10	<10
[DR + LFA-3]-liposomes (3 μ l)	<10	1,910	510
[DR + LFA-3]-liposomes (1 μ l)	<10	1,480	215

5×10^4 hybridoma cells were cultured for 24 h with irradiated JY cells (10^4 cells/well), HLA-DR, LFA-3, or [DR + LFA-3]-containing liposomes (3 or 1 μ l) as indicated. Culture supernatants were assayed for the presence of IL-2 as described in Materials and Methods. Liposomes were also prepared as described in Materials and Methods.

did not stimulate IL-2 production (data not shown). Thus, stimulation appeared to require the presence of both HLA-DR, the ligand for the TCR, and LFA-3.

Monoclonal antibody inhibition of the response of 16.CD2-15 to [DR + LFA-3]-liposome stimulation was compared with that of JY cell stimulation (Fig. 2). Both anti-CD2 (TS2/18) and anti-LFA-3 (TS2/9) mAb substantially inhibited IL-2 production in response to both stimuli. Similarly, mAb directed against monomorphic determinants of HLA-DR (LB3.1) inhibited both stimulations. However, mAb directed against murine MHC class I antigen H-2k^k (100.30.3) did not inhibit IL-2 production. These data demonstrated that stimulation of IL-2 production was dependent upon expression of CD2 on the T cell, and upon the LFA-3 and HLA-DR antigens expressed on the stimulator cell or in liposomes.

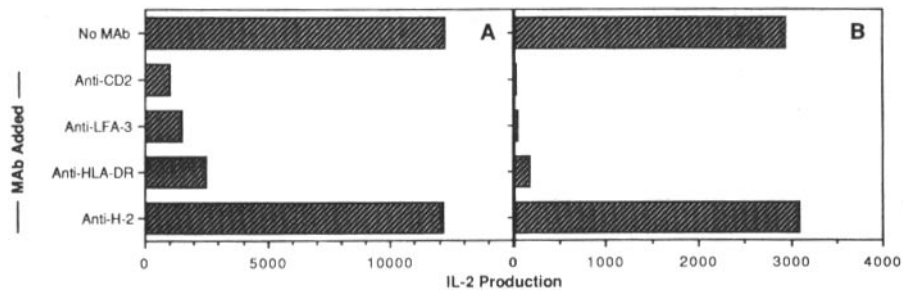


FIGURE 2. Inhibition of antigen and [DR + LFA-3]-liposome stimulation of IL-2 production. 5×10^4 CD2⁺ hybridoma cells (16.CD2-15) were cultured with 10^5 irradiated (100 cGy from a cesium source) JY stimulator cells (A), or 3 μ l [DR + LFA-3]-liposomes (B), as described in Materials and Methods, in the presence or absence of mAb, as indicated. The anti-CD2 mAb TS2/18 and anti-LFA-3 mAb TS2/9 were used at a 1:1,200 dilution of ascites fluid. The anti-HLA-DR mAb LB3.1 and anti-H-2k^k mAb 100.30.3 were used at a 5% final concentration of culture supernatant. Data are presented as units of IL-2 activity per milliliter as described in Materials and Methods. Note difference in scale of horizontal axis.

Expression and Function of CD2 Δ B Molecules in the Murine T Cell Hybridoma. The CD2/LFA-3 interaction may participate in antigen-dependent T cell stimulation by increasing adhesion of the stimulator cell to the T cell and/or by providing a signal required for T cell activation. LFA-3 incorporated into [DR + LFA-3]-liposomes may allow adhesion to CD2 on the T cell, promoting the interaction of the TCR with HLA-DR antigens. To determine whether the CD2/LFA-3 interaction participates in cell-cell adhesion and provides an activation signal, we have constructed a cytoplasmic deletion cDNA mutant of CD2, CD2 Δ B, in which the COOH-terminal 100 amino acids of CD2 have been replaced with a serine (Fig. 3). 155.16 was infected with a defective retrovirus that imparts resistance to G418 and surface expression of CD2 Δ B. G418-resistant cell lines were analyzed by indirect immunofluorescence for CD2 Δ B expression. Eight cell lines that expressed the murine TCR and mutant CD2 Δ B molecules at levels equivalent to the wild-type CD2 $^+$ hybridomas were chosen for further study, an example of which is shown in Fig. 4. The expression of the murine TCR, assessed by indirect immunofluorescence using F23.1, an mAb specific for the V β 8 region of the TCR, was equivalent on the parent hybridoma, the CD2 $^+$ hybridoma 16.CD2-15, and the hybridoma expressing the deletion mutant 16.CD2 Δ B-7 (Fig. 4). Indirect immunofluorescence of the CD2 $^+$ and CD2 Δ B $^+$ hybridomas using a panel of anti-CD2 mAb was equivalent (Fig. 4 and data not shown). The expression of the LFA-1 molecule was equivalent on all cell lines (data not shown).

Eight hybridomas expressing the CD2 Δ B molecule were analyzed. All eight CD2 Δ B $^+$ cell lines were able to produce equivalent amounts of IL-2 as the wild type CD2 $^+$ hybridomas upon stimulation with phorbol-12-myristate-13-acetate and the calcium ionophore ionomycin (data not shown). No CD2 Δ B $^+$ hybridoma responded to combinations of stimulatory pairs of anti-CD2 mAb, such as 9.6 or 9-1 (Table II and data not shown). Four CD2 Δ B $^+$ T cell hybridomas showed no enhanced responsiveness to JY stimulation, and four deletion mutants showed a slight enhancement of IL-2 production (1.5-2-fold) in comparison to the parent 155.16 (Table II and data not shown). Of the eight CD2 Δ B $^+$ hybridomas, the most responsive cell line, 16.CD2 Δ B-7, was analyzed for LFA-3 adhesion. 16.CD2 Δ B-7 retains the ability to bind purified LFA-3 comparably to the wild type CD2 $^+$ hybridoma. Scatchard analysis of the binding of a soluble form of LFA-3 to the hybridomas expressing CD2 and the CD2 Δ B mutants showed that both had a K_d of 30 nM (A. Peterson, manuscript in preparation).

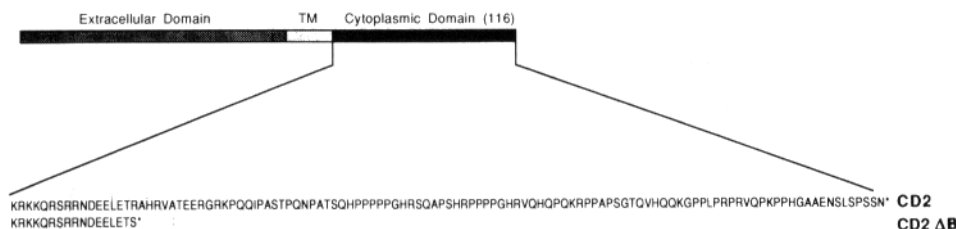


FIGURE 3. Schematic diagram of CD2 and CD2 Δ B. Beneath the diagram of the CD2 molecule, the amino acids of the cytoplasmic domain of the CD2 and CD2 Δ B molecules are shown. The COOH-terminal 100 amino acids of CD2 are replaced by a serine in the CD2 Δ B molecule. TM, transmembrane.

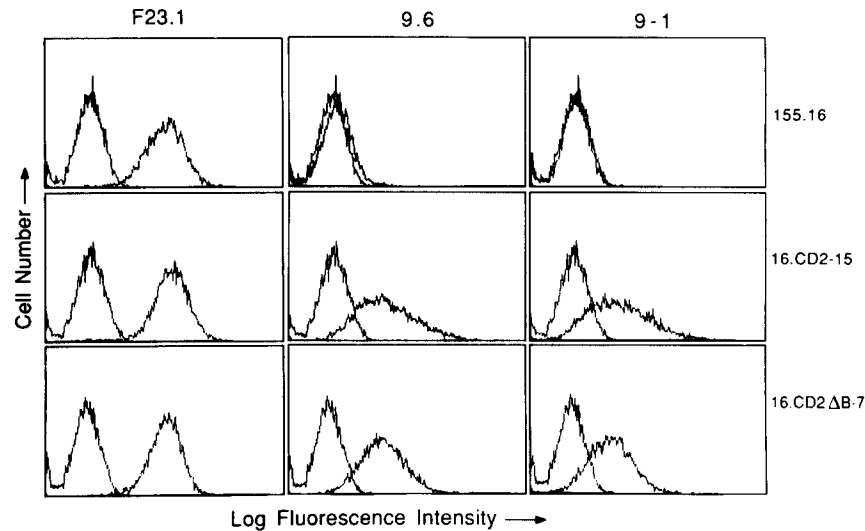


FIGURE 4. Expression of the murine TCR and human CD2 molecules by the murine T cell hybridomas. Representative flow cytometric histograms are shown for the parent hybridoma 155.16, the CD2⁺ hybridoma 16.CD2-15, and the CD2ΔB⁺ hybridoma 16.CD2ΔB-7. Hybridomas were stained with saturating concentrations of mAb directed against the TCR (F23.1) and CD2 (9.6, 9.1) molecules, followed by incubation with FITC-GAM as described in Materials and Methods. The negative controls were cells stained with FITC-GAM alone.

Since the hybridoma 16.CD2ΔB-7 was able to bind LFA-3 but unable to be stimulated by pairs of anti-CD2 mAb, stimulation with [DR + LFA-3]-liposomes was then assessed. Unlike the hybridomas expressing the wild type CD2 protein, the CD2ΔB⁺ did not respond to [DR + LFA-3]-liposomes (Table III), demonstrating

TABLE II
Stimulation of CD2⁺ and CD2ΔB⁺ Hybridomas

Exp. 1 Stimulant	IL-2 production				
	155.16	16.CD2-15	16.CD2-18	16.CD2ΔB-7	16.CD2ΔB-14
	<i>U/ml</i>				
None	<10	<10	<10	<10	<10
JY cells	410	>2,000	1,230	815	160
9.6 + 9-1 mAb	<10	825	240	<10	<10
MT110 + 9-1 mAb	<10	1,440	415	<10	<10
Exp. 2 Stimulant	155.16	16.CD2-15	16.CD2-43	16.CD2ΔB-7	16.CD2ΔB-8
None	<10	<10	<10	<10	<10
JY cells	100	>2,000	420	210	150
MT110 + 9-1	<10	905	300	<10	<10
MT910 + 9-1	<10	890	220	<10	<10

5×10^4 hybridoma cells were cultured for 24 h with irradiated JY cells (10^4 cells/well), or with mAb as indicated. 9.6, MT110, MT910, and 9-1 mAb were used at a final concentration of 1 μ g/ml. Incubation of the hybridomas with each anti-CD2 mAb alone did not stimulate IL-2 production (data not shown). Culture supernatants were assayed for the presence of IL-2 as described in Materials and Methods.

TABLE III
*Stimulation by LFA-3 and HLA-DR Liposomes of Murine
 T Cell Hybridomas*

Stimulant	IL-2 production			
	155.16	16.CD2-15	16.CD2-43	16.CD2ΔB-7
	<i>U/ml</i>			
None	<10	<10	<10	<10
JY cells	260	7,395	1,210	540
DR liposomes	<10	12	<10	<10
LFA-3 liposomes	<10	<10	<10	<10
DR + LFA-3-liposomes	<10	7,810	505	<10

5×10^4 hybridoma cells were cultured for 24 h with liposomes (5 μ l) or JY cells (10^5 cells/well) as indicated. Cultured supernatants were assayed for the presence of IL-2 as described in Materials and Methods. HLA-DR, LFA-3, and [DR + LFA-3]-liposomes were also prepared as described in Materials and Methods.

that adhesion of LFA-3 to CD2 alone was insufficient for activation, and that the cytoplasmic domain was required for LFA-3 stimulation through the CD2 molecule.

Discussion

In this report, we have demonstrated that murine T cell hybridomas expressing the human CD2 molecule produce IL-2 in response to stimulation with purified LFA-3 and HLA-DR incorporated into liposomes. LFA-3 may participate in antigen-dependent T cell stimulation via CD2 by promoting adhesion of the target or stimulator cell to the T cell and/or by providing an activating signal to the T cell. In immune interactions, adhesion precedes antigen recognition and T cell activation, and these functions are therefore complementary. Stimulation of CD2⁺ hybridomas by liposomes containing both LFA-3 and HLA-DR proteins would be consistent with either role, since LFA-3 adheres to CD2 on the T cell and may allow the TCR to interact with HLA-DR antigens more efficiently. Like antigen stimulation, the [DR + LFA-3]-liposome stimulation of the CD2⁺ hybridomas may be inhibited by mAb directed against CD2, LFA-3, or HLA-DR.

The CD2ΔB⁺ hybridomas expressing a cytoplasmic deletion mutant of CD2 bind LFA-3 comparably to wild-type CD2, but are ineffective in allowing stimulation with [DR + LFA-3]-liposomes. The K_d of CD2ΔB expressed in the hybridoma for LFA-3 is the same as that of wild-type CD2. Furthermore, the CD2ΔB and wild-type CD2 molecules transfected into COS cells both promote adhesion to sheep erythrocytes, which express the sheep homologue of LFA-3 (A. Peterson, unpublished). If adhesion alone were responsible for CD2-mediated stimulation of the hybridoma's response to antigen, then this mutant should be as effective as wild-type CD2. However, no response is elicited by [DR + LFA-3]-liposomes. Thus, in this system, it appears that a predominant effect of the CD2/LFA-3 interaction is to provide an intracellular signal that synergizes with the signal provided by stimulation of the TCR-CD3 complex. This conclusion is supported by the response of the CD2ΔB⁺ hybridomas to incubation with intact JY cells, in which there was, at most, a slight enhancement of IL-2 production compared to the parent hybridoma. This enhancement could be attributed to the more limited role of adhesion.

We have previously shown that a hybridoma expressing a point mutant of CD2, Q51L CD2, with a single amino acid substitution at position 51 of glutamine for leucine, did not react with the 9.6 mAb, but had retained binding to and could be stimulated to produce IL-2 to other anti-CD2 mAb with 9-1 mAb (18). The Q51L CD2⁺ hybridomas did not respond to sheep erythrocytes nor murine L cells transfected with and expressing human LFA-3 plus 9-1 mAb (18), demonstrating that the Q51L CD2 molecule is unable to use LFA-3 for activation. Unlike the hybridomas expressing the wild type CD2 molecule, the Q51L CD2⁺ hybridomas did not exhibit enhanced IL-2 production in response to antigen-specific JY stimulation (18). These findings support the conclusion that the inability of Q51L CD2⁺ hybridomas to demonstrate an enhanced response to JY cell stimulation is due to the inability to bind LFA-3 expressed on the stimulator cell. The Q51L CD2⁺ hybridoma did not respond to stimulation with [DR + LFA-3]-liposomes (data not shown). These data provide further evidence that the enhanced response observed with the wild type CD2⁺ hybridoma is specific and depends upon the ability of LFA-3 to bind to CD2.

We (18) and others (16) have shown that LFA-3 binding to CD2, in the presence of one anti-CD2 mAb, induces antigen-independent T cell activation. Furthermore, the CD2/LFA-3 interaction may affect T cell stimulation via the TCR-CD3 pathway. Resting human T cells may be stimulated to proliferate by one anti-CD3 mAb in the presence of murine L cells expressing human LFA-3 (17) or by an anti-CD2 mAb (9-1) (3). Two signals are necessary to stimulate T cells by the CD2 pathway. T cell function may be triggered by pairs of anti-CD2 mAb (1-3), or by one anti-CD2 mAb in the presence of either an anti-CD3 mAb (3) or LFA-3 (16, 18). LFA-3 is widely expressed on nucleated cells (9); however, the interaction of CD2 with LFA-3 alone is insufficient for stimulation. Given the necessity for antigen to regulate the T cell immune response in the periphery, the second signal for T cell stimulation may be provided not through CD2, but through the TCR interacting with specific antigen. The CD2/LFA-3 interaction may provide an accessory function to augment a weak antigen-driven response. In this report, we have shown that T cells may be activated by purified LFA-3 binding to CD2 and the TCR interacting with its ligand, and that these signals are synergistic for the T cell. These findings extend previous data on the biological role of the interaction of LFA-3 with CD2, and of the interrelationship of CD2 and the TCR. The signal provided by binding LFA-3 to CD2 alone is insufficient for activation but may contribute to antigen-dependent T cell activation. A second signal, here provided by the TCR interacting with its ligand, is required for T cell activation. Whether a quaternary complex formed among CD2 and the TCR-CD3 complex on the T cell, and LFA-3 and antigen/MHC on the stimulator cell, allows for potentiation of these signals remains to be determined.

Summary

T cells may be activated either by the antigen-specific T cell receptor (TCR)-CD3 complex or the cell surface receptor CD2. A natural ligand for CD2 has been found to be lymphocyte function-associated antigen 3 (LFA-3), a widely distributed cell surface glycoprotein. To investigate the interaction of these two pathways, we have expressed the cDNA encoding the human CD2 molecule in a murine T cell hybridoma that produces IL-2 in response to HLA-DR antigens. Expression of the CD2 mole-

cule markedly enhances IL-2 production in response to LFA-3⁺ antigen-bearing stimulator cells, and this stimulation is inhibited by anti-CD2 and anti-LFA-3 mAb. To further define the role of LFA-3 in antigen-dependent T cell activation, we have studied the ability of the purified ligands of CD2 and the TCR to stimulate the hybridoma. Neither liposomes containing purified HLA-DR antigens nor liposomes containing purified LFA-3 were able to stimulate the parent or the CD2⁺ hybridoma. However, liposomes containing both purified LFA-3 and HLA-DR, the physiological ligands for CD2 and the TCR, respectively, stimulate IL-2 production by the CD2⁺ but not the parent hybridoma, suggesting that complementary interactions between the TCR-CD3 complex and the CD2 pathway may regulate lymphocyte activation.

To determine whether the CD2/LFA-3 interaction participates in cell-cell adhesion and provides an activation signal, we have constructed a cytoplasmic deletion mutant of CD2, CD2 Δ B, in which the COOH-terminal 100 amino acids of CD2 have been replaced with a serine. Hybridomas expressing the CD2 Δ B molecule were examined. Deletion of the cytoplasmic domain of CD2 did not alter binding of LFA-3 but eliminated the ability of CD2 to increase the response of the hybridoma to liposomes containing both HLA-DR and LFA-3, demonstrating that adhesion of LFA-3 to CD2 alone was insufficient for activation, and that the cytoplasmic domain was required for LFA-3 stimulation through the CD2 molecule. T cells may be activated by purified LFA-3 binding to CD2 and the TCR interacting with its ligand, and these signals appear to be synergistic for the T cell. These results suggest that the CD2/LFA-3 interaction not only plays a role in cell-cell adhesion but provides a stimulatory signal for T cell activation.

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