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An Anti-CD2 Monoclonal Antibody That Both Inhibits and Stimulates T Cell Activation Recognizes a Subregion of CD2 Distinct from Known Ligand-Binding Sites

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The T lymphocyte glycoprotein CD2 appears to have an important role in human T cell development and activation. A novel anti-CD2 monoclonal antibody, designated UMCD2, was shown to block E rosetting, and therefore was defined as recognizing the $T11_1$ ligand-binding epitope. Binding of UMCD2 to T cells and thymocytes was blocked by several, but not all, anti-$T11_1$ antibodies, suggesting that the $T11_1$ epitope consists of more than one subepitope. In functional studies, the combination of UMCD2 plus anti-$T11_3$ was mitogenic for T cells; in some individuals, the level of activation was as high as that seen for the combination of anti-$T11_2$ plus anti-$T11_3$. However, when UMCD2 was added to other stimuli mitogenic for T lymphocytes, such as IL-2 or anti-CD3–Sepharose, it inhibited T cell responses. Although the combination of UMCD2 and anti-$T11_3$ induced an increase in cytoplasmic free calcium, the inhibitory activities of UMCD2 were not accompanied by effects on calcium fluxes. A panel of previously characterized CD2 mutants was then analyzed for binding of UMCD2 and other anti-CD2 monoclonals. Surprisingly, UMCD2 bound to all mutants tested, although the other anti-CD2 antibodies with specificity for the ligand-binding region of CD2 each failed to bind to one or more mutants. These data suggest that binding of antibody to a particular CD2 epitope can have opposite effects on the state of T cell activation, depending on the costimulus. Moreover, inhibitory effects mediated through CD2 may use a signaling mechanism distinct from that used in CD2 pathway activation. Of particular interest, the portion of the CD2 ligand-binding region recognized by UMCD2 is distinct from areas of the CD2 molecule that have previously been studied.

INTRODUCTION

Human T lymphocytes were initially enumerated by their capacity to form rosettes (E rosettes) with sheep erythrocytes. The CD2 (T11) receptor which mediates binding to sheep erythrocytes is a 50-kDa pan-T cell surface protein that can also mediate activation of T cells and thymocytes (1).

Monoclonal antibodies have been used to define multiple epitopes on the CD2 antigen; three of these epitopes have been designated $T11_1$, $T11_2$, and $T11_3$. The $T11_1$ and $T11_2$ epitopes are found on the surfaces of thymocytes and both resting and activated T cells, but the $T11_3$ epitope is masked on resting T cells. The $T11_1$ epitope is distinguishable from the $T11_2$ because only anti-$T11_1$ antibodies block E rosetting. The combination of anti-$T11_2$ plus anti-$T11_3$ antibodies is highly mitogenic for mature

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T cells (1). This combination induces IL-2 receptor expression on thymocytes but not IL-2 production, and thus only is mitogenic for thymocytes when used in conjunction with IL-2 or other costimuli (2). A natural ligand of CD2, CD58 (LFA-3), binds to the $T11_1$ epitope and has comitogenic effects on T cells. This ligand is present on functionally important cell types that interact with T cells, such as thymic epithelial cells and monocytes (3–6).

In this report, a new anti-$T11_1$ antibody, termed UMCD2, is described. This antibody, in contrast to many others, is shown to be capable of stimulating T cells and thymocytes when used in combination with anti-$T11_3$. However, it inhibits T cell activation in response to other stimuli (lectin, anti-CD3, or IL-2). Binding of UMCD2 to T cells and thymocytes was inhibited by some but not all of the other anti-$T11_1$ antibodies tested. Moreover, UMCD2 recognizes a portion of the CD2 molecule not yet defined by mutants that affect ligand or antibody binding. We conclude that the $T11_1$ epitope can be subdivided into multiple different domains; the domain identified by UMCD2 can mediate bidirectional effects on T cell activation.

MATERIALS AND METHODS

Antibodies

The monoclonal antibody UMCD2 (IgG_{2a}-κ) was generated against a T cell line (ST-1) by previously described methods (7). Monoclonal antibodies 9.6 and 9-1 were a gift from Carl June (8); OKT3 and OKT11 hybridomas were obtained from American Type Culture Collection (Rockville, MD); other anti-CD2 antibodies and the IgM anti-CD3 antibody 2AD2A2 were generous gifts of Drs. Stuart Schlossman and Ellis Reinherz (Dana Farber Cancer Inst., Boston, MA).

Isolation of Lymphoid Cells

Peripheral blood mononuclear cells (PBMC) were obtained from healthy human volunteers and isolated from venous blood by centrifugation for 25 min at 1300*g* over Ficoll–Paque (Pharmacia, Gaithersburg, MD). Thymocytes were prepared from fresh normal thymus of pediatric patients undergoing corrective cardiac surgery. A single-cell suspension was obtained as described (2).

Mitogenic Effects of UMCD2

Assays were performed on triplicate cultures in 96-well U-bottomed plates (Costar, Cambridge, MA) with 50,000 cells in a final volume of 0.2 ml medium/well. The medium was RPMI 1640 supplemented with 10% (v/v) fetal calf serum, 2 m*M* glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. PMA (1 ng/ml), PHA (0.5 μg/ml), IL-2 (16 U/ml; generous gift of Cetus Corp., Emeryville, CA), or the designated ascites (1/500 or 1/1000 dilutions unless otherwise indicated) was added to selected wells. The cultures were pulsed at 72 hr with 0.8 μCi/well [^{3}H]TdR (New England Nuclear, Boston, MA), harvested 18 hr later, and counted in a liquid scintillation counter.

Competitive Binding Assay

Fresh thymocytes were incubated first with a 1/250 dilution of antibodies against epitopes of CD2 followed by a 1/100 dilution of FITC-conjugated UMCD2 (prepared

as described (2)) and analyzed by flow cytometry using a Coulter EPICS C cell sorter. The percentage inhibition of UMCD2 binding was calculated by comparison of the percentage of cells positive above background fluorescence with the percentage positive after incubation with UMCD2-FITC alone.

Western Blotting

Western blotting was performed as previously described (7). Briefly, a membrane fraction was prepared from a T cell clone and solubilized in a detergent-containing buffer. Some aliquots of the lysates were precleared by incubation with OKT11-conjugated Sepharose or with UMCD2-conjugated Sepharose for 1 hr at 4°C. The samples were subjected to centrifugation, and the supernatants were loaded onto a 7.5% acrylamide gel in the absence of reducing agents, followed by electrophoretic transfer to nitrocellulose. Strips of nitrocellulose were probed with ascites at 1/100 dilutions, followed by ^{125}I-labeled sheep anti-mouse Ig (Fab fragment; Amersham Corp., Arlington Heights, IL), and dried strips were subjected to autoradiography as previously described (7).

Calcium Flux Studies

PBMC isolated from healthy human volunteers as described above or Jurkat cells were suspended at a concentration of 20×10^6 cells/ml in culture medium (RPMI 1640 supplemented with 10% fetal calf serum, 2 m*M* glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin). Cells were loaded with the dye indo-1 (Molecular Probes, Eugene, OR) at a concentration of 1.5 μM for 25 min at 37°C in the dark (9). Cells were washed three times and resuspended in culture medium. Aliquots of 10^6 cells were held on ice until subjected to flow cytometry. The ratio of violet to blue fluorescence was determined as a function of cell number; the change in mean ratio was used as a measure of the extent of flux. Readings were taken at the indicated times following addition of antibodies, PHA, or ionomycin. When two stimuli were added, the interval between addition of the first and second stimulus was less than 1 min.

Expression of CD2 Mutants and Analysis of Transfectants

A panel of mutated cDNAs encoding altered forms of CD2, which have been previously described (10), were generously provided by Dr. Brian Seed. Plasmids were transformed into competent *Escherichia coli* MC1061/P3 and grown overnight in liquid culture, and then isolated by a scaled-up version of the alkaline lysis miniprep procedure (11). After extensive digestion with Xho 1, the uncut and cut bands were analyzed on 1% agarose gels to verify the expected molecular weight of the CD2 cDNA insert.

Transfections were performed in 100-mm tissue culture dishes containing 750,000 COS 7 cells per dish with 10 ml DME supplemented with 10% FBS medium. The cultures were plated overnight and transfected the following day in the presence of DEAE-dextran. After a $2\frac{1}{2}$-hr incubation, the cells were washed and shock treated with PBS/10% DMSO for 2 min, and then washed and fed with DME/10% FBS. The cells were then incubated at 37°C for 72 hr and stained with anti-CD2 monoclonal antibodies, followed by fluoresceinated goat anti-mouse immunoglobulin. Samples were then analyzed by flow cytometry using a Coulter Elite or Epics C instrument.

Dead cells were gated out using 0.2 mg propidium iodide added to each sample. Fluorescence histograms were obtained on a 5000 cell count.

In each set of transfection experiments the CDM 8 vector without a CD2 insert was used as a negative control, and a plasmid containing the wild-type CD2 cDNA was used as a positive control.

RESULTS

UMCD2 Recognizes the CD2 (T11) Antigen

UMCD2 stained all T lymphocytes, greater than 95% of thymocytes, and all T cell clones, but was not reactive with various non-T cell populations (data not shown). To confirm that UMCD2 recognizes the CD2 antigen, a Western blot was performed (Fig. 1). A total membrane fraction was prepared from a T cell clone, solubilized, and subjected to SDS–polyacrylamide gel electrophoresis followed by transfer to nitrocellulose. The nitrocellulose was incubated with UMCD2 (lane 1) or with OKT11 (lane 4). Control ascites detected no bands on the nitrocellulose (data not shown), whereas both OKT11 and UMCD2 recognized a single band with a molecular weight of 50 kDa. Aliquots of the lysate were precleared either with OKT11–Sepharose (lanes 2 and 5) or with UMCD2–Sepharose (lanes 3 and 6). Preclearing with OKT11 almost entirely removed all UMCD2-reactive material; consistent with this result, preclearing with UMCD2 removed all OKT11-reactive material. A single band at approximately 50 kDa was also obtained by immunoprecipitation of lysates of ^{125}I-labeled cells (data not shown). These data indicate that both antibodies recognize nonconformational epitopes of CD2 that are preserved during SDS–PAGE and blot transfer procedures.

UMCD2 Is an Anti-T11$_1$ Antibody

Other anti-CD2 antibodies have been defined as recognizing one of three epitopes, designated T11$_1$, T11$_2$, and T11$_3$. Those antibodies which are directed against the T11$_1$ epitope inhibit the formation of E rosettes. When UMCD2 was tested, it likewise

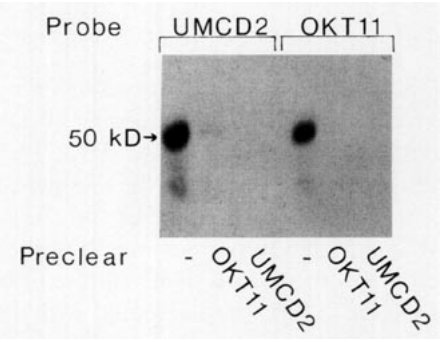


FIG. 1. Immunoblotting of CD2 by UMCD2 and OKT11. Membrane fractions were prepared from a T cell clone and subjected to Western blotting as described under Materials and Methods. Antibody probes were: lanes 1–3, UMCD2 ascites, 1/100 dilution; lanes 4–6, OKT11 ascites, 1/50 dilution. Lanes 2 and 5 contain samples which were precleared with OKT11–Sepharose beads; lanes 3 and 6 contain samples which were precleared with UMCD2–Sepharose beads. Immunoblotting with control ascites showed no bands (data not shown).

TABLE 1

Comparison of UMCD2 with Other Anti-CD2 Antibodies

Antibody	Epitope	Inhibition of E rosetting[a]	Inhibition of UMCD2 binding[b]
Control	–	–	–
UMCD2	$T11_1$	+	+
OKT11	$T11_1$	+	+
3PT2H9	$T11_1$	+	+
7T47E10	$T11_1$	+	+
9.6	$T11_1$	+	+
7T47A9	$T11_1$	+	–
3T48B5	$T11_1$	+	–
ROLD21H8	$T11_1$	+	–
1OLD24C1	$T11_2$	–	–
1MONO2A6	$T11_3$	–	–
9-1	$T11_3$	–	–

[a] + designates greater than 90% inhibition of E rosette formation as compared to medium alone; – designates less than 10% inhibition.

[b] Inhibition of UMCD2 binding was determined by a competitive binding assay (see Materials and Methods). Positive (+) levels of inhibition were greater than 96% at 1/1000 dilutions of blocking antibody; negative (–) levels were less than 1% inhibition of UMCD2 binding.

was found to inhibit E rosetting (Table 1), suggesting that UMCD2 is an anti-$T11_1$ antibody. To confirm these results, UMCD2 was compared to a variety of other anti-CD2 antibodies in a competitive binding assay. Fresh thymocytes (Table 1) or PBMC (Fig. 2) were incubated in the presence of one of the anti-CD2 antibodies and then washed and stained with a directly fluoresceinated UMCD2. Samples were analyzed by flow cytometry. Table 1 shows that 1OLD24C1 (anti-$T11_2$), 1MONO2A6 (anti-$T11_3$), and 9-1 did not inhibit binding of UMCD2 to thymocytes. However, OKT11, 3PT2H9 (1), 7T47E10 (1), and 9.6, all of which block E rosetting, did inhibit binding of UMCD2, suggesting that these antibodies recognize the same epitope. Surprisingly, 7T47A9 (1), 3T48B5 (2), and ROLD21H8, all of which have also previously been

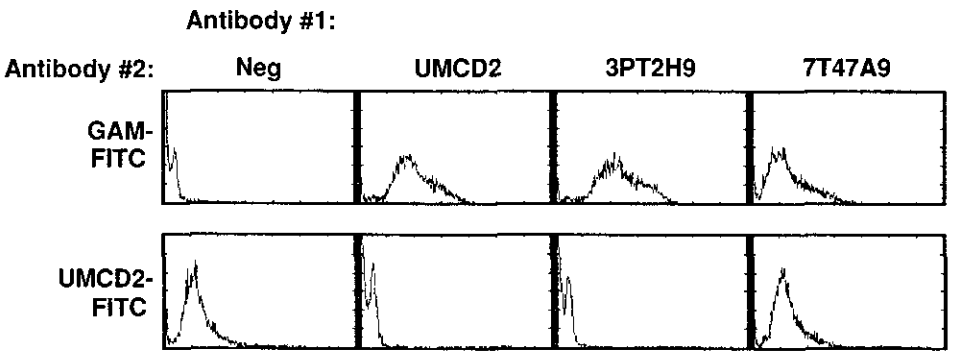


FIG. 2. Selective inhibition of UMCD2 binding by specific anti-$T11_1$ antibodies. PBMC (Fig. 2) and thymocytes (data not shown) were isolated as described under Materials and Methods, stained with the indicated antibodies followed by a FITC-conjugated second antibody, and analyzed by flow cytometry.

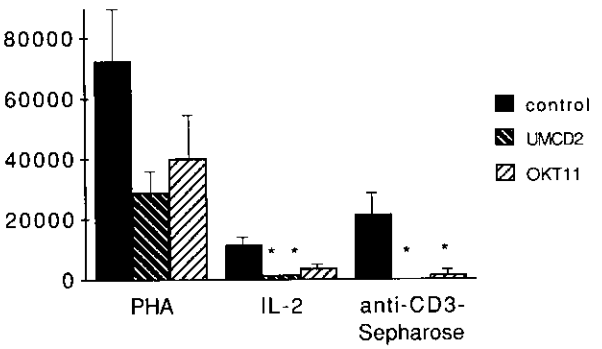


FIG. 3. Inhibition of T cell proliferation by UMCD2. Proliferation assays were performed as described under Materials and Methods. Additions to the culture medium were as follows: 0.5 μg/ml PHA, 16 U/ml IL-2, or anti-CD3–Sepharose at a dilution of 1/100. Data are represented as the means of 11 separate experiments for IL-2 stimulation, or 6 separate experiments for the other stimuli, with standard error indicated. P values were determined by a paired t test. $^{*}P < 0.05$; $^{**}P < 0.005$. The P values not indicated were $0.1 > P > 0.05$.

defined as anti-$T11_1$ antibodies (1), did not inhibit binding of UMCD2 (Fig. 2 and Table 1). Similar results were obtained with E^+ cells (data not shown). Thus, UMCD2 discriminates subepitopes of $T11_1$.

Effects of UMCD2 on T Cell Proliferation

We next sought to compare the effects of UMCD2 on PBMC which were incubated in the presence of mitogens with the effects of other anti-CD2 monoclonal antibodies. Figure 3 shows that UMCD2 inhibited the mitogenic effects of other stimuli, such as PHA, OKT3–Sepharose, and IL-2. For example, a 1/1000 dilution of UMCD2 reduced proliferation of PBMC in response to IL-2 to approximately 15% of the levels induced by IL-2 alone. Protein A-purified UMCD2 had inhibitory effects on T cell responses comparable to a 1/250 dilution of ascites, at concentrations between 0.2 and 5 μg/ml (data not shown). OKT11 had similar effects, although the extent of inhibition of proliferation was somewhat less than observed with UMCD2.

TABLE 2

Stimulation of PBMC Proliferation by UMCD2 plus Anti-$T11_3$

	[^{3}H]Thymidine incorporation (cpm)	
Experiment number	UMCD2 + anti-$T11_3$	Anti-$T11_2$ + anti-$T11_3$
1	38,425 ± 2,279	113,820 ± 13,048
2	2,463 ± 2,560	43,653 ± 7,637
3	34,430 ± 7,772	51,640 ± 5,005
4	20,846 ± 3,802	18,992 ± 950
5	21,626 ± 2,654	19,808 ± 8,950

Note. Results are expressed as the mean of triplicate cultures ± standard deviation and are the net cpm above background.

Control experiments performed using combinations of anti-CD2 antibodies showed that, as previously observed (1), combinations of anti-$T11_2$ and anti-$T11_3$ antibodies were strongly mitogenic for PBMC, while combinations of anti-$T11_1$ and anti-$T11_2$ antibodies were not (Table 2 and data not shown). Surprisingly, the combination of UMCD2 (an anti-$T11_1$) plus anti-$T11_3$ was mitogenic for PBMC, although the level of stimulation was somewhat variable compared to $T11_2$ plus $T11_3$ (Table 2).

Costimulation of Thymocytes by UMCD2

UMCD2 alone did not augment thymocyte responses to PMA or to IL-2 (Table 3). In contrast, the combinations of UMCD2 plus anti-$T11_3$ and anti-$T11_2$ plus anti-$T11_3$ were strongly mitogenic in the presence of PMA or IL-2, although the combination of UMCD2 plus anti-$T11_2$ was not. Thus, thymocytes, like PBMC, respond strongly to either UMCD2 or anti-$T11_2$ in combination with anti-$T11_3$. As observed previously (2), thymocytes, unlike PBMC, only respond to the combinations of anti-CD2 antibodies when a costimulus such as IL-2 or PMA is supplied.

UMCD2 Effects on Calcium Flux

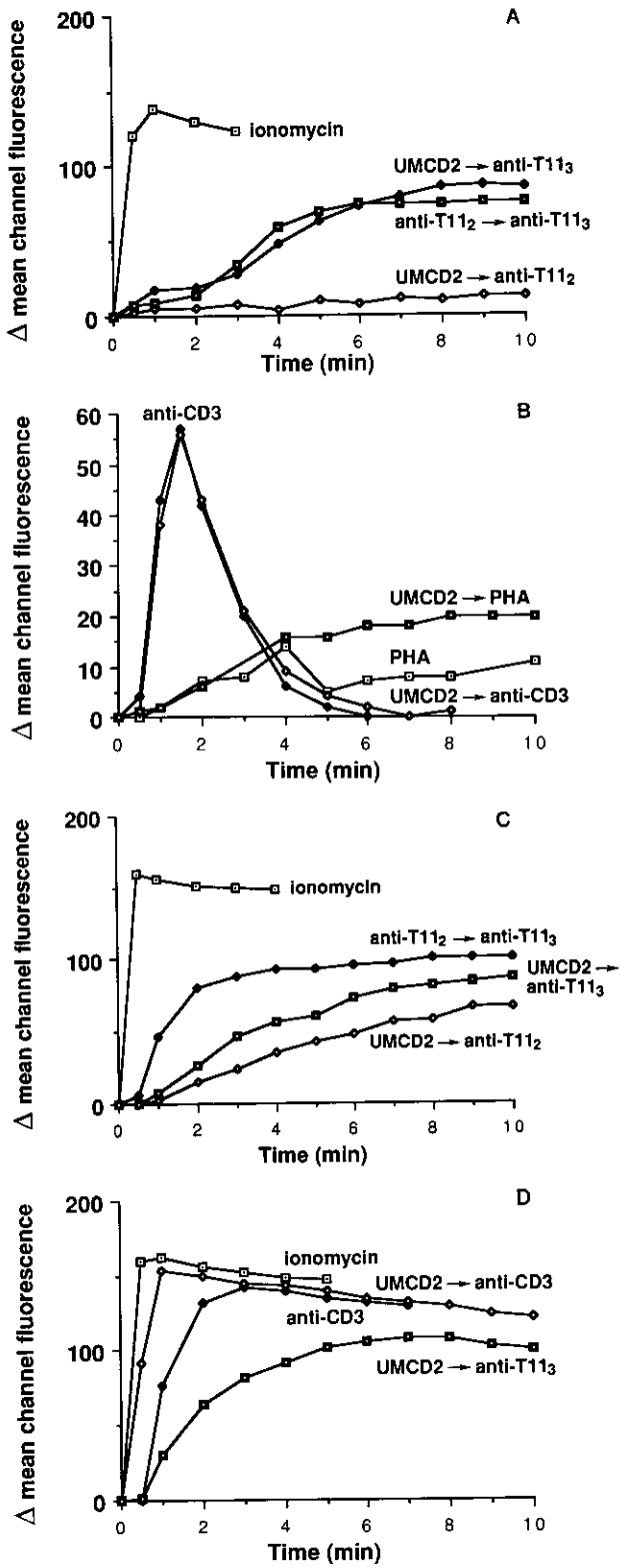
Experiments were performed to test whether the inhibitory effects of UMCD2 on certain proliferative stimuli could be explained by effects on proximal signal transduction events that result in calcium fluxes. Cells were loaded with indo-1, a calcium-binding dye, and changes in intracellular calcium levels were measured by flow cytometry. An increase in the violet to blue ratio (in Fig. 4, measured as a change in mean channel fluorescence) indicates an increase in intracellular free calcium. The calcium ionophore ionomycin was included as a positive control for calcium flux. Figure 4A shows that, for PBMC, UMCD2 plus anti-$T11_3$ caused a calcium flux similar to the flux obtained with the combination of anti-$T11_2$ plus anti-$T11_3$. UMCD2 plus anti-$T11_2$ had very little effect on calcium flux. These calcium flux data correlate with the mitogenic effects of these combinations of antibodies. To determine if the inhibitory effect of UMCD2 on other mitogenic stimuli is characterized by an inhibition of calcium flux, UMCD2 and either PHA or anti-CD3 were added to PBMC, and the calcium flux measured. Figure 4B shows that UMCD2 did not inhibit the calcium

TABLE 3

Comitogenic Effects of UMCD2 on Thymocytes

Antibody	[^{3}H]Thymidine incorporation (cpm)		
	Medium	PMA	IL-2
None	342 ± 60	2,476 ± 1,589	6,004 ± 1,667
UMCD2	398 ± 110	1,061 ± 208	2,483 ± 191
Anti-$T11_2$	321 ± 37	4,762 ± 5,441	4,369 ± 615
Anti-$T11_3$	342 ± 24	793 ± 145	6,650 ± 1,870
UMCD2 + anti-$T11_2$	118 ± 29	582 ± 53	1,629 ± 687
UMCD2 + anti-$T11_3$	257 ± 144	136,968 ± 45,493	84,276 ± 3,409
Anti-$T11_2$ + anti-$T11_3$	809 ± 275	158,109 ± 9,759	62,735 ± 831

Note. Results are expressed as the mean of triplicate cultures ± standard deviation.


A
200
100
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Δ mean channel fluorescence
ionomycin
UMCD2 → anti-T11$_3$
anti-T11$_2$ → anti-T11$_3$
UMCD2 → anti-T11$_2$
0 2 4 6 8 10
Time (min)
B
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anti-T11$_2$ → anti-T11$_3$
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anti-CD3
UMCD2 → anti-T11$_3$
0 2 4 6 8 10
Time (min)

flux induced by the addition of anti-CD3 and may even have augmented the low calcium flux induced by PHA. Thus, we conclude that the inhibitory effects of UMCD2 are not transduced through the calcium pathway. Figures 4C and 4D show that generally similar results were obtained with the Jurkat leukemic T cell line. Of interest, however, anti-$T11_2$ plus UMCD2 induced a calcium flux in Jurkat cells, a response not seen in PBMC.

Analysis of UMCD2 Binding to CD2 Mutants

A panel of CD2 mutants spanning amino acids 45–54 and 91–100 was analyzed in a transient transfection system. These mutants have previously been shown to alter antibody and/or ligand binding to the CD2 molecule. As shown in Table 4, all mutations tested affected binding of one or more anti-$T11_1$ monoclonals, with little or no effect on anti-$T11_2$ and anti-$T11_3$ (1OLD24C1 and 1MONO2A6) antibody binding. Unexpectedly, UMCD2 bound equally well to all CD2 mutant proteins and to wild-type CD2. Thus, UMCD2 recognizes a portion of the CD2 molecule distinct from recognition sites of other anti-$T11_1$ antibodies, including those antibodies that can block UMCD2 binding. Representative flow cytometry histograms from these experiments are shown in Figure 5.

DISCUSSION

We have raised an anti-CD2 antibody, UMCD2, which recognizes the $T11_1$ epitope. However, this antibody is distinct from other anti-$T11_1$ antibodies in that it is capable both of inhibiting T cell proliferation, (in the absence of anti-$T11_3$), and of inducing or augmenting proliferation (in the presence of anti-$T11_3$). For PBMC, UMCD2 markedly inhibited responses to not only IL-2 and anti-CD3–Sepharose (Fig. 3), but also alloantigen and nominal antigen (data not shown). Functional effects of UMCD2 on T cell responses were observed with both purified antibody and ascites, indicating that nonspecific effects of ascites did not account for the findings. Other anti-CD2 antibodies either stimulate T cell activation or block T cell responses (as seen with OKT11 in previous work and in the present experiments), but the potent bidirectional functional effects of UMCD2 appear to be unique.

It has previously been observed that anti-$T11_1$ antibodies may be mitogenic in combination with either anti-$T11_2$ or anti-$T11_3$, but not both (8). However, these experiments were done in the presence of a submitogenic dose of 12-*O*-tetradecanoylphorbol 13-acetate (TPA). UMCD2 did not generally require this additional costimulus in the presence of anti-$T11_3$, and furthermore was not comitogenic with anti-$T11_2$, even in the presence of PMA.

The fact that a single anti-CD2 antibody has apparently opposite effects on T cell function depending on the nature of the concurrently present T cell stimulatory factors

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FIG. 4. UMCD2 effects on calcium fluxes in PBMC and Jurkat cells. Cells were loaded with the calcium-binding dye indo-1 as described under Materials and Methods. Antibodies, ionomycin, or PHA were then added as indicated. The ratio of blue to violet fluorescence was measured as a function of cell number. The values are plotted as the difference in mean channel fluorescence from baseline. Each graph represents a separate experiment. A and B, PBMC; C and D, Jurkat cells. In B, anti-CD3 refers to 2AD2A2 (see Materials and Methods); in D, anti-CD3 refers to OKT3. Dilutions: A, 1 μg/ml ionomycin; 1/500 ascites. B, C, and D, 5 μg/ml ionomycin, 15 μg/ml PHA, 1/100 ascites.

TABLE 4

Analysis of CD2 Mutants

	Asp 45	Lys 46	Lys 47	Lys 48	Ile 49	Ala 50	Gln 51	Phe 52	Arg 53	Lys 54	UMCD2	OKT11	7T47E10	9.6	3PT2H9	7T47A9	2T48B5	1OLD24C1	1MON02A6
Wild-type											+	+	+	+	+	+	+	+	+
Mutant #1				Thr			Glu				+	-	+		+	+	+	+	+
Mutant #2		Asn		Asn							+	-	+				+	+	+
Mutant #3		Asn		Asn				Leu			+	-	+				+	+	+
Mutant #4			Arg				Lys				+		-				+/-		
Mutant #5		Met		Asn			His				+		-				+		
Mutant #6							Arg	Ser			+		-				+		
Mutant #7	Asn						Thr				+	+	+				+		
Mutant #8				Met			Arg				+	-	-	-	+	-			
Mutant #9							Arg				+	+/-	-	-	+	+/-	+		
Mutant #10							Leu				+	+/-	+/-		+	+/-	+/-		
Mutant #11							Arg	Leu			+	+/-	-		+	-	-		
Mutant #12				Thr							+	-	+/-		-	+	+		
Mutant #13				Gln							+	+/-	+	-	+	+/-	+		
Mutant #14				Met							+	+/-	-		+	+/-	+		+
Mutant #15							Arg	Leu			+	+	-		+	-	+	+	+
Mutant #16					Gln						+	-	-	-	+/-	-	+		
Wild-type	Tyr 91	Asp 92	Thr 93	Lys 94	Gly 95	Lys 96	Asn 97	Val 98	Leu 99	Glu 100									
Mutant #17							Lys			Lys	+				+		-		
Mutant #18		Ala									+	-	+	+	+	+/-			
Mutant #19		Val									+	-	+	+	+	+	+		
Mutant #20	Asp										+	-	+	+	+	+/-	-		
Mutant #21									Ser		+	-	+	+	+	+	-		
Mutant #22	Cys										+	+/-	+/-	+	+	+			
Mutant #23		His									+	-	+	+	+	+	+		+
Mutant #24			Ser		Val		Ile				+	-	+	+/-	+	+	-		+
Mutant #25	Asp										+	-	+	+/-	+	+	-		+
Mutant #26					Glu						+	-	+	+	+	+	+		
Mutant #27					Glu		Lys		Ser		+	-	+	+/-	+	+/-	+/-		+/-
Mutant #28		Tyr							Phe		+	-	+		+	+		+	+
Mutant #29	Thr							Ile		Lys	+	-	+		+	+	-	+	+

Note. Spaces, not determined; +, binding >30% positive; −, not binding <10% positive; ±, reduced binding 20–30% positive.

suggests that the role of CD2 in regulating T cell function is quite complex and probably bidirectional. The mechanism of the inhibitory effects of UMCD2 is not clear, but could reflect linkage of the CD2 molecule to more than one signal transduction pathway (12). Although the stimulatory effect of anti-CD2 antibodies is mediated by the calcium flux, the inhibitory effect of UMCD2 alone does not correlate with a reduction in the calcium flux. This suggests that UMCD2 may affect a different signaling step not directly associated with calcium fluxes, in exerting its inhibitory effects. Lack of effect on calcium fluxes is consistent with inhibition of T cell responses to IL-2 (as well as lectin and anti-CD3), since signaling through the IL-2 receptor does not involve a calcium flux. It has previously been suggested that inhibitory anti-CD2 mAbs inhibit IL-2R expression and not IL-2 synthesis. T cells that were treated with anti-CD3 plus an anti-CD2 antibody were prevented from attaining usual levels of anti-CD3-induced IL-2R expression; those cells that did synthesize IL-2Rs were capable of responding normally to IL-2. These data suggest that the anti-CD2-induced inhibition of mitogenesis occurs at a step between the initial calcium flux and induction of IL-2R synthesis (13–15).

The blocking studies shown in Table 1 indicate that the $T11_1$ "epitope" actually consists of more than one antibody binding site. Other studies are consistent with these results (10, 16, 17), and with possible functional differences between subregions of $T11_1$ (17). Experiments by Peterson and Seed (10) suggest that three of the antibodies that block binding of UMCD2 (3PT2H9, 7T47E10, and 9.6) bind to the same region of CD2, while one of the antibodies that does not block UMCD2 binding (3T48B5) binds to a different region. However, extensive analysis of CD2 mutants shown in

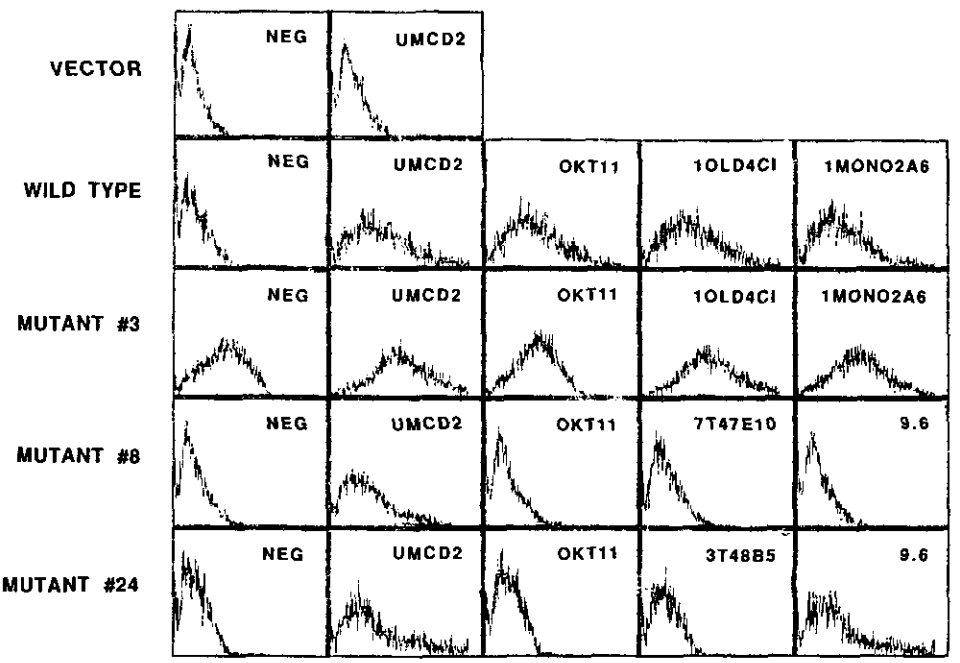

FIG. 5. Flow cytometric analysis of transfectants expressing CD2 or representative CD2 mutants. Experiments were performed as described under Materials and Methods. Background fluorescence gates were reset for each transfectant. The antibodies used are indicated on each panel.

Table 4 indicates that binding of some anti-T11$_1$ antibodies can be affected by alteration in both amino acid 45–54 and amino acid 91–100 regions of the CD2 sequence. A recent report suggests that one of these regions may also be involved in binding of CD59, a second ligand for CD2 (18). It is of particular interest that UMCD2 appears to bind to neither of these regions, suggesting that an as yet unidentified region of CD2 may also be involved in ligand binding. This is likely to be a linear sequence, in view of the good binding of UMCD2 on reduced Western blots. The unusual bidirectional functional effects of UMCD2 suggests that this putative novel ligand-binding domain may be of particular functional importance.

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