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Immunology

At least two non-antigen-binding molecules are required for signal transduction by the T-cell antigen receptor

(calcium/inositolphospholipids/signaling/mutants/complementation)

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ABSTRACT **In the T-cell somatic mutant J.CaM1, the T-cell antigen receptor complex is poorly coupled to the inositolphospholipid second messenger system; some antibodies against the invariant CD3 subunit of the receptor retain their agonist function in J.CaM1. Here we show by a combination of complementation assays that the mutation in J.CaM1 affects a molecule other than the antigen-binding Ti subunit, suggesting that Ti is coupled indirectly to the signal transduction apparatus through a pathway involving the CD3 complex. We also describe another mutant, J.CaM2, in which the receptor complex is completely uncoupled from inositolphospholipid hydrolysis. J.CaM2 defines an additional complementation group, suggesting that signal transduction by the antigen receptor depends on at least two molecules distinct from Ti.**

The T-cell antigen receptor (CD3–Ti), consisting of a clonotypic heterodimer (Ti) and a complex of invariant integral membrane proteins (CD3) (1), is among the large class of surface receptors that are coupled to the metabolism of inositolphospholipids for the generation of intracellular second messengers (2). Many monoclonal antibodies (mAbs) reactive with Ti or CD3 proteins elicit production of second messengers derived from phosphatidylinositol-4,5-bisphosphate and subsequent elevation of intracellular free Ca^{2+} (1). In addition, an inducible tyrosine kinase activity has been demonstrated during receptor-mediated activation (3).

To identify molecules required for signal transduction competence by CD3–Ti, we have described a system for isolating and characterizing T-cell somatic mutants with impaired transmembrane signaling (4). The receptor complex of the mutant J.CaM1, which is expressed at normal levels, fails to demonstrate inositolphospholipid metabolism or detectable Ca^{2+} mobilization in response to anti-Ti mAbs and some anti-CD3 mAbs (4). However, several individual anti-CD3 mAbs, as well as a combination of nonagonist anti-Ti and anti-CD3 mAbs, are capable of eliciting second messenger production in J.CaM1 cells (4, 5). The partial integrity of receptor function in J.CaM1 cells led to the conclusion that the mutation most likely resides in a proximal component of the signaling pathway.

Somatic hybridization experiments demonstrated that the mutation in J.CaM1 cells is recessive (5). Since allelic exclusion makes the Ti genes functionally hemizygous, they are statistically likely candidates for single-hit mutations resulting in functional effects. However, the present complementation studies using gene transfer and a heterokaryon assay demonstrate that the mutation resides in a non-Ti molecule. A second mutant, J.CaM2, expresses high levels of antigen receptor that appear to be entirely devoid of signal transduction function through the inositolphospholipid system. The mutation in J.CaM2 cells also is recessive and affects a molecule distinct from the Ti α chain, the Ti β chain, and the defective molecule in J.CaM1 cells.

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METHODS

Cell Lines. The human leukemic T-cell line Jurkat (clone E6-1) and its derivative J.RT3-T3.5 were maintained as described (6). PEER (7), MOLT-13 (7), HPB-ALL (8), and CEM (9) were maintained similarly. J-α-3.4 was derived and maintained as described (10). J.CaM1 was isolated as described (4). J.CaM2 was isolated from an irradiated Jurkat subclone (200 rad of γ radiation from ^{137}Cs; 1 rad = 0.01 Gy) by a modified selection procedure (see *Results*).

mAbs. mAb C305 recognizes an idiotype expressed on the Jurkat Ti (6). mAb A2B4 reacts with the Ti α chain expressed on murine 2B4 cells (10). mAbs 235 (11) and OKT3 (12) recognize CD3 determinants. mAb W6/32 (13) recognizes an invariant determinant expressed on human HLA class I antigens.

Flow Cytometry and Fluorimetry. Cell surface immunofluorescence techniques were performed as described (6). The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was assessed by fluorimetry (2, 4) and by flow cytometry (4) with Indo-1. For analysis of heterokaryons (see below), cells were exposed to a 364-nm excitation beam for Indo-1 and a 501-nm beam for fluorescein (fluorescein emission detected through a 530-nm band-pass filter). Electronic gates excluded cells lacking Indo-1 fluorescence.

Heterokaryon Formation. Cells were alternately loaded with Indo-1 (6 μM) or stained with mAb W6/32 (anti-HLA mAb) followed by directly conjugated fluorescein isothiocyanate-goat anti-mouse immunoglobulin. Cells were washed in serum-free RPMI 1640 (SFM), and 2×10^6 cells of each partner were centrifuged together in a 24-well (16 mm) plastic tissue culture plate to form a monolayer. The medium was aspirated, and 300 μl of 50% (vol/vol) polyethylene glycol (EM Science, Cherry Hill, NJ) in SFM was added gently at room temperature to cover the monolayer. After 90 sec, the cells were gently washed with SFM and resuspended in 2 ml of RPMI 1640 supplemented with 10% (vol/vol) fetal calf serum. The cells were incubated for 1 hr at 37°C and then analyzed as described above.

DNA Transfection. DNA-mediated gene transfer into J.CaM1 cells was performed by electroporation. Approximately 10^7 cells were washed and resuspended in 1 ml of sterile buffer (20 mM Hepes, pH 7.05/137 mM NaCl/5 mM KCl/0.7 mM Na_2HPO_4/6 mM dextrose) containing 20 μg of the designated plasmid. A custom-built Ekker-type electroporation device was used to discharge an electric field pulse (225 V) through the cell suspension by way of platinum electrodes with a path length of 0.4 cm and capacitance of 1080 μF, at room temperature. After 10 min the sample was

Abbreviations: mAb, monoclonal antibody; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration.

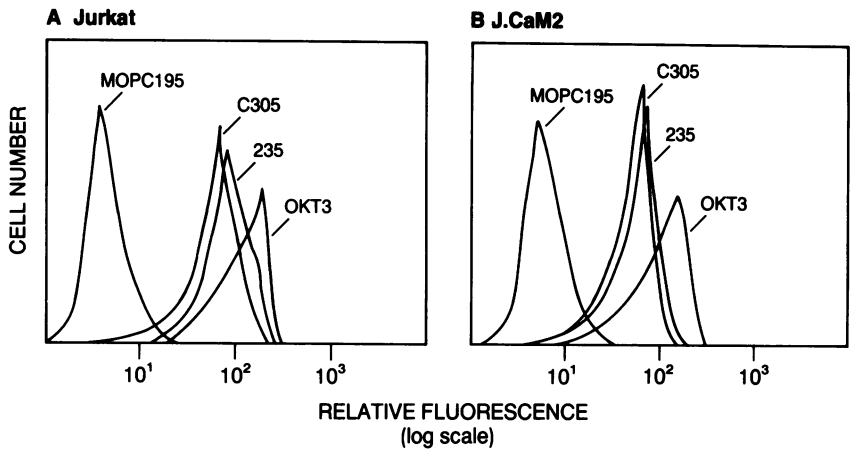


FIG. 1. Immunofluorescence profiles of Jurkat and J.CaM2. Jurkat (*A*) and J.CaM2 (*B*) cells were stained with the indicated mAb and analyzed by flow cytometry. MOPC195, negative control; OKT3 and 235, anti-CD3 mAbs; C305, mAb reactive with Jurkat Ti β chains.

diluted with culture medium. After a 2-day culture, the cells were selected in G418 (GIBCO) at 2 mg/ml. After 2–3 weeks, viable colonies were screened by immunofluorescence, cloned by limiting dilution, and then reassayed for expression.

Other Methods. Inositol phosphates were quantitated by anion-exchange chromatography essentially as described (2). Cloning by limiting dilution and cell surface radioiodination and immunoprecipitation analysis were performed as described (6).

RESULTS

To isolate a signal-transduction somatic mutant with a distinct phenotype, we utilized the knowledge that some anti-CD3 mAbs are effective agonists for J.CaM1, the first mutant isolated (4, 5). In a modified form of the selection protocol used for deriving J.CaM1 cells (4), an irradiated Jurkat subclone was subjected to lectin-mediated growth retardation followed by alternating sorts for receptor-bearing cells and for those cells that failed to mobilize Ca^{2+} in response to mAb 235, an anti-CD3 mAb (11) that is an agonist for J.CaM1 cells (4). One mAb 235-binding clone (J.CaM2) failed to elevate $[Ca^{2+}]_i$ upon exposure to mAb 235 (see below). J.CaM2 was assessed by immunofluorescence and flow cytometry for reactivity with anti-CD3–Ti mAbs (Fig. 1). No appreciable differences were observed in the immunofluorescence profiles of Jurkat and J.CaM2 cells upon staining with mAbs C305 (anti-Ti), 235 (anti-CD3), or OKT3 (anti-CD3) (Fig. 1), or with any other anti-CD3–Ti mAbs evaluated (Leu4, L142, L143, WT31, R140; data not shown). Similarly, $NaDodSO_4$/PAGE and isoelectric focusing electrophoresis revealed no substantial differences in the electrophoretic properties of the Ti α or β chains, or the CD3 γ, δ, or ε chains in Jurkat and J.CaM2 cells (data not shown).

Fluorimetry studies demonstrated no change in $[Ca^{2+}]_i$ in J.CaM2 cells in response to a saturating dose of mAb 235, in contrast to the large increase in parental cells (Table 1). Additionally, J.CaM2 showed no response to mAb C305, to mAb OKT3, or to mAbs C305 plus OKT3 (Table 1). Likewise, in contrast to Jurkat, J.CaM2 showed no increase in intracellular levels of inositol trisphosphate or its metabolites at the time of peak induction of inositol phosphates in Jurkat (Table 1). Therefore, in J.CaM2, CD3–Ti is completely uncoupled from the phosphatidylinositol 4,5-bisphosphate second messenger system in response to all mAbs tested thus far.

An important step in analyzing the signal transduction mutants is to identify the affected molecules. The phenotype of J.CaM1 and the hemizygosity of Ti genes led to the hypothesis that the mutation in J.CaM1 affects a Ti chain. As a direct approach to complementation, we used gene transfer to introduce a cDNA encoding a heterologous Ti α chain gene into J.CaM1 to attempt to restore signaling function. This protein, the Ti α subunit from the murine T-cell hybridoma 2B4, associates functionally with the Ti β subunit expressed by Jurkat, even in competition with the endogenous Jurkat Ti α chain (10). The expression plasmid p2B4αFneo, containing this murine Ti α chain cDNA, was therefore introduced stably into Jurkat (10) and into J.CaM1.

Surface expression of the 2B4 α and endogenous Ti chains was assessed by immunofluorescence and flow cytometry by using the mAbs A2B4, which reacts specifically with the 2B4 α chain (10), and C305, which reacts specifically with the Ti β chain of Jurkat (ref. 10; A.W., unpublished observations). Transfected Jurkat (J-α-3.4) and transfected J.CaM1 (J.CaM1-2B4α-1.1) cells expressed high levels of C305- and A2B4-reactive proteins (Fig. 2 *B* and *D*). In fluorimetry studies, J-α-3.4 cells responded to mAb C305 and to mAb A2B4 (Table 2), demonstrating that expression of the 2B4 α chain does not impair the functioning of the endogenous Ti β chain and that the heterologous α chain itself can mediate signal transduction in the wild-type Jurkat cells. In contrast, J.CaM1-2B4α-1.1 was unresponsive either to mAb C305 or to mAb A2B4 (Table 2). Therefore, introduction of a functional Ti α chain does not reconstitute signaling responsiveness to C305, and this chain itself is not functional in the context of J.CaM1. This finding provides compelling evidence that the mutation in J.CaM1 does not lie in the α subunit of Ti.

Attempts to express a heterologous Ti β chain in J.CaM1 failed to achieve sufficient levels of cell surface expression to

Table 1. Ca^{2+} mobilization and inositol phosphate production in J.CaM2

	Peak $[Ca^{2+}]_i$, nM				$InsP_3$, % of basal level		$InsP + InsP_2$, % of basal level	
Cell	235	C305	OKT3	C305 + OKT3	235	C305	235	C305
Jurkat	972	843	705	ND	407 ± 12	341 ± 2	671 ± 14	610 ± 1
J.CaM2	92	96	108	108	104 ± 17	110 ± 12	90 ± 1	104 ± 1

Jurkat and J.CaM2 cells were analyzed by fluorimetry. Peak $[Ca^{2+}]_i$ values after exposure to the mAb (1:1000 dilution of ascites) indicated are shown. The basal level of $[Ca^{2+}]_i$ in Jurkat is 127 ± 29 nM (mean ± SD, n = 4) and in J.CaM2 is 91 ± 9 nM (n = 4). Inositol phosphates were quantitated after stimulation for 20 min. Inositol trisphosphate ($InsP_3$) and combined inositol bisphosphate ($InsP_2$) and inositol monophosphate ($InsP$) values are expressed relative to unstimulated cells (mean ± SEM, n = 3).

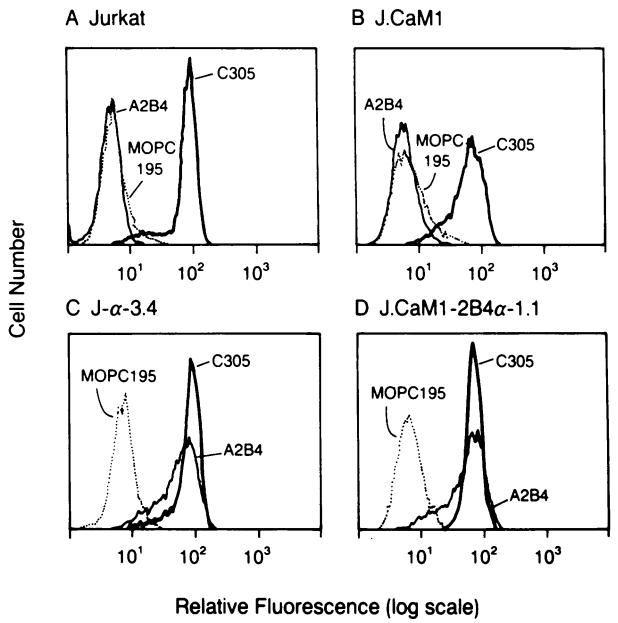


FIG. 2. Immunofluorescence profiles of Ti α chain transfectants. Jurkat (*A*), J.CaM1 (*B*), J-α-3.4 (*C*), and J.CaM1-2B4α-1.1 (*D*) cells were stained with mAb C305 to detect endogenous Ti β chains and mAb A2B4 to detect the transfected Ti α chains isolated from 2B4 cells. MOPC195, negative control; A2B4, mAb reactive with 2B4 Ti α chains; C305, mAb reactive with Jurkat Ti β chains.

assess whether or not this molecule restored function. We, therefore, developed a heterokaryon assay that permits assessment of signal transduction shortly after fusion to various partners. One partner cell line was loaded with Indo-1, and the second partner was labeled with fluorescein-conjugated antibody against a surface marker, HLA class I antigens. The populations of cells were fused and kept at 37°C for 1 hr prior to flow cytometry; incubation for only 15 min

Table 2. Ca^{2+} mobilization responses in Ti α-chain transfectants

Cell	$[Ca^{2+}]_i$, nM			
	C305		A2B4	
	Basal	Peak	Basal	Peak
Jurkat	99	1923	76	76
J-α-3.4	67	1393	62	704
J.CaM1	64	82	65	65
J.CaM1-2B4α-1.1	62	122	58	58

Representative basal (unstimulated) and peak $[Ca^{2+}]_i$ values after stimulation with either mAb (1:1000 dilution of ascites) are presented.

yielded identical results. Aliquots of cells were then analyzed by multicolor immunofluorescence and flow cytometry, in which three populations of cells are detectable: Indo-1-loaded partner 1 cells, fluorescein-positive partner 2 cells, and fluorescein-positive Indo-1-loaded heterokaryons. We gated exclusively on the cells containing Indo-1 and thus could compare the Ca^{2+} mobilization response in unfused partner 1 cells with the response of heterokaryons in the same sample. Under these conditions, staining of the HLA molecules does not cause mobilization of cellular Ca^{2+} nor does it restore the responsiveness of either mutant to mAb C305 (see Figs. 3*D* and 5).

We first assessed the ability of heterokaryons between J.CaM1 loaded with Indo-1 and fluoresceinated parental Jurkat cells to respond to C305. The Ca^{2+}-mobilization responses of the unfused J.CaM1 cells (lower quadrant, fluorescein-negative) and the heterokaryons (upper quadrant, fluorescein-positive) are shown (Fig. 3*A*). After exposure to C305 for 1 min, the J.CaM1 cells demonstrated no substantial change in the Indo-1 ratio. In contrast, the majority of the heterokaryons demonstrated a marked increase, indicating a substantial rise in $[Ca^{2+}]_i$ (Fig. 3*B*). Thus, the defective signaling in J.CaM1 is recessive in the heterokaryon assay. It was important to determine whether the

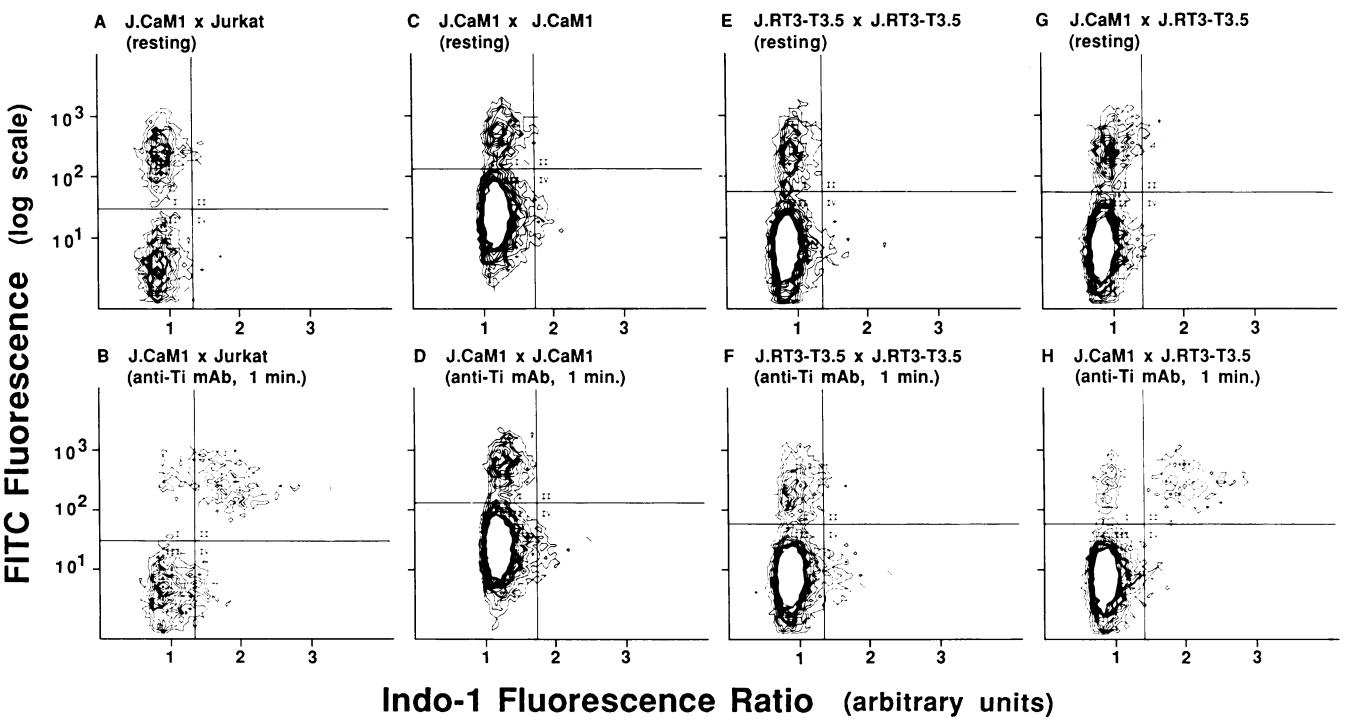
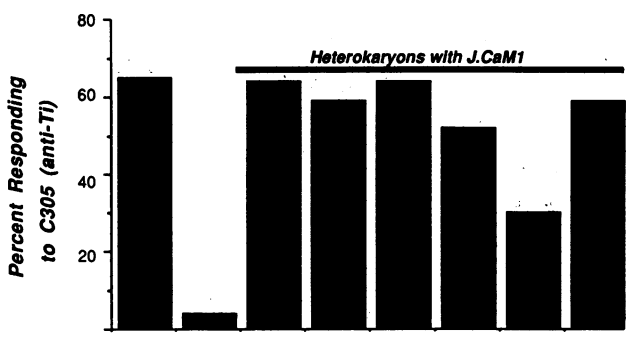


FIG. 3. Heterokaryon complementation analysis. J.CaM1 cells (*A–D*, *G*, and *H*) or J.RT3-T3.5 cells (*E* and *F*) were loaded with Indo-1. Jurkat (*A* and *B*), J.CaM1 (*C* and *D*), or J.RT3-T3.5 cells (*E–H*) were stained with anti-HLA mAb (W6/32). After fusion of the indicated partners, the Indo-1 ratio of resting cells (*A*, *C*, *E*, and *G*) was compared to the ratio 1 min after stimulation with C305 (*B*, *D*, *F*, and *H*). FITC, fluorescein isothiocyanate.

mutation in J.CaM1 causes the signaling phenotype as a result of a quantitative decrease in expression of a molecule that influences signal transduction. Heterokaryons formed between J.CaM1 cells that were loaded with Indo-1 and J.CaM1 cells that were fluorescein-tagged showed no increased responsiveness (Fig. 3 *C* and *D*). Therefore, fusion itself is not responsible for restoration of function. Additionally, it does not appear that the mutation exerts its effect through a partial decrease in expression of a component that can be restored to necessary levels by homotypic fusion.

It was then possible to assess complementation by specific T-cell partners with known phenotypes. The Jurkat-derived mutant cell line J.RT3-T3.5 fails to express full-length Ti β transcripts or protein, preventing expression of CD3–Ti on the cell surface (14). Homotypic fusion of this cell caused no substantial change in its ability to mobilize Ca^{2+} in response to C305 (Fig. 3 *E* and *F*). Fusion of J.CaM1 cells to J.RT3-T3.5 cells restored its responsiveness to C305 (Fig. 3 *G* and *H*). Although the Ti β chain expression defect in J.RT3-T3.5 may be complemented in this fusion, it is highly unlikely that in this time frame formation of the heterokaryon permitted appreciable reexpression of Ti β protein from this cell. Therefore, reconstitution of J.CaM1 likely requires the presence of a non-Ti β chain signal transduction component that is either absent or defective in J.CaM1 but that is expressed in J.RT3-T3.5 cells.

As a quantitative measure of reconstitution, we determined the fraction of heterokaryons in the basal $[Ca^{2+}]_i$ electronic window that have left that window after stimulation with C305 for 1 min, the optimal time after stimulation to assess responsiveness. By this criterion J.CaM1 cells were reconstituted comparably by fusion with Jurkat and J.RT3-T3.5 cells (Fig. 4). Fusion with HPB-ALL cells also reconstituted responsiveness of J.CaM1 to C305. Heterokaryons formed between J.CaM1 and three T-cell lines that lack either Ti α chains (CEM and PEER) or lack both Ti α and β chains (MOLT-13) all responded significantly to C305, demonstrating that neither of the Ti chains is required for reconstitution of J.CaM1.



Phenotype of Fusion Partner								
Ti-α mRNA	+	+	+	+	+	−	−	−
Ti-β mRNA	+	+	+	+	−	+	−	+
Surface CD3	+	+	+	+	−	−	+	+
C305-Binding	+	+	+	−	−	−	−	−

FIG. 4. Summary of responses of heterokaryons between J.CaM1 and various partners. Fraction of cells in the electronic window representing resting $[Ca^{2+}]_i$ that moves to the window representing elevated $[Ca^{2+}]_i$ by 1 min after stimulation with C305. Responses of Jurkat and J.CaM1 cells are compared to those of heterokaryons between J.CaM1 and partners whose CD3–Ti phenotypes are summarized. +, Expressed in partner; −, not expressed in partner.

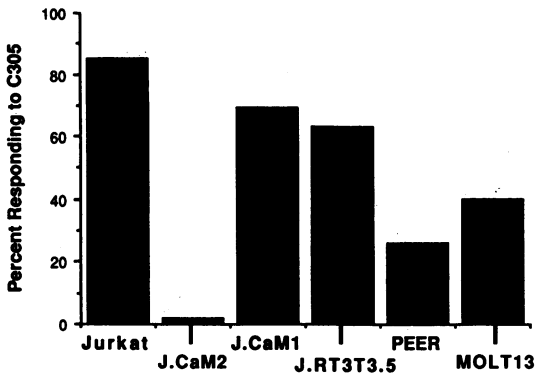


FIG. 5. Summary of responses of heterokaryons between J.CaM2 and various partners. Responses to C305 by heterokaryons formed between J.CaM2 and the following cell lines: Jurkat, J.CaM2, J.CaM1, and other cells characterized in Fig. 4.

The heterokaryon complementation assay makes possible rapid assignment of a mutant to a known or new complementation group. Heterokaryons between J.CaM2 and Jurkat cells demonstrated a wild-type Ca^{2+}-mobilization response to C305 (Fig. 5), indicating that the mutation behaves recessively in this assay. Interestingly, heterokaryons between J.CaM2 and J.CaM1 also revealed a wild-type response (Fig. 5), demonstrating that the mutations in these two mutants lie in separate complementation groups. In addition, heterokaryons between J.CaM2 and T cells lacking Ti α chains (PEER), Ti β chains (J.RT3-T3.5), or both Ti α and β chains (MOLT-13) also responded to C305, indicating that the mutation in J.CaM2 does not affect either of these chains (Fig. 5). In sum, these complementation studies with J.CaM1 and J.CaM2 cells define at least two molecules distinct from the Ti α and β chains that are required for transmembrane signaling by the T-cell antigen receptor.

DISCUSSION

We have used our previously reported method (4) to isolate two somatic mutants from the leukemic cell line Jurkat, named J.CaM1 and J.CaM2, each with distinct deficiencies in transmembrane signaling despite the presence of the receptor. In J.CaM1 the pattern of selective responsiveness to certain stimuli and the knowledge that Ti genes are allelically excluded suggested that the mutation might reside in a Ti protein. Conventional somatic cell hybridization has demonstrated that the mutation was recessive and could be complemented when the appropriate components were present (5). We, therefore, used gene transfer to introduce a wild-type Ti α chain gene into J.CaM1. Stimuli reactive with either the endogenous Ti β chain or the transfected Ti α chain failed to elicit transmembrane signaling in the J.CaM1 transfectant, although both stimuli elicited substantial responses in a Jurkat transfectant. These results indicate that the Ti α chain is not the locus of the defect in J.CaM1.

In attempts to perform similar studies with heterologous Ti β chains, adequate cell surface expression of the transfected gene was not obtained. Instead we developed a short-term reconstitution assay in which analysis by flow cytometry allowed changes in $[Ca^{2+}]_i$ to be monitored in parental cells and in heterokaryons. These studies suggested that the mutation in J.CaM1 affects a molecule distinct from Ti α and β chains, since cells specifically lacking either (or both) chain reconstituted the Ca^{2+} responses of J.CaM1. Although formally it is possible that one or more of the Ti β chain-negative partner cells expressed the silent Ti β chain gene upon fusion to J.CaM1, the rapidity of the complementation (15 min) makes this possibility seem unlikely. Moreover, one such partner (MOLT-13) would require accurate Ti β chain gene

rearrangements involving the Jurkat Ti β chain variable region gene segment in a large proportion of cells to produce a functional Ti β chain reactive with mAb C305, an unlikely event. Finally, both PEER and HPB-ALL cells express endogenous Ti β chains that are not recognized by the stimulating mAb C305, ensuring that in these heterokaryons the response to C305 is mediated by the β chain contributed by J.CaM1. Therefore, these complementation experiments suggest that reconstitution of J.CaM1 requires a component distinct from the Ti subunit.

The fact that anti-CD3 mAbs in some model systems elicit signal transduction events comparable to those of anti-Ti mAbs has been the sole basis for the assumption that the CD3 complex performs the transmembrane signaling function for Ti. It has remained a formal possibility that CD3 has another unknown function but that its physical association with Ti allows mAbs directed against CD3 indirectly to induce Ti conformational changes that are then translated into the usual Ti-mediated second messenger events. The present results argue strongly against this possibility and suggest that the CD3 complex is indeed coupled directly to the second messenger apparatus. This conclusion derives from the observation that many CD3 mAbs are agonists for J.CaM1, while normal Ti chains in J.CaM1 are unable to mediate responses to usual Ti agonists (4, 5). Since the defect in signal transmission is distal to Ti, CD3 would be unlikely to be able to promote second messenger production through Ti in J.CaM1. Instead, it now seems likely that the vectorial flow of information in receptor-mediated activation is indeed from Ti to CD3, and from CD3 to the next component. We have speculated that the role of CD3 is as a signal transduction amplifier, increasing either the magnitude or the diversity of intracellular signals that result from binding of antigens to Ti (4).

Like J.CaM1, J.CaM2 expresses normal levels of CD3–Ti on its cell surface, and no gross alterations in conformation or composition are detectable by immunofluorescence, $NaDodSO_4$/PAGE, or isoelectric focusing. Unlike J.CaM1, J.CaM2 is unresponsive to any mAbs assessed to date, including those that are agonists for J.CaM1. This failure of J.CaM2 to mobilize Ca^{2+} is the result of uncoupling from phosphatidylinositol-4,5-bisphosphate hydrolysis. J.CaM2 lies in a new complementation group distinct from that of J.CaM1 and from those of the Ti α and β chains. Thus, Ti depends on at least two non-Ti molecules for signal transduction competence.

In the heterokaryon assay the lost function is restored, but the precise genetics of the mutation in J.CaM2 are not known. For example, the mutation might actually lie in a molecule that does not itself contribute to the signal transduction process but that affects the expression of another molecule that is involved directly in signaling. In this case, the effects of a dominant mutation might not be detected in the transient assay. In J.CaM1, permanent somatic hybrids were used (5) to determine unambiguously that the mutation in this cell is recessive.

An important question still to be answered is where the mutations in J.CaM1 and J.CaM2 reside. One hypothesis is that a different CD3 protein is mutated in each of the two mutants. Several reports have demonstrated that rapid receptor turnover occurs even in the resting state (15, 16), implying that recycling Ti subunits conceivably could associate with awaiting intracellular pools of CD3 molecules provided even by T cells lacking CD3 on the cell surface. Some support for the T-cell specificity of the mutated molecules comes from the failure of a variety of non-T cells substantially to restore function to the mutants (data not shown). However, since quantitative extinction of wild-type receptor function occurs in such heterokaryons with non-T cells, these results are inconclusive. Alternative hypotheses include a guanine nucleotide-binding regulatory protein and phospholipase C as candidate mutant molecules.

A spontaneous mutant derived from the murine T-cell hybridoma 2B4 was described in which one component of the CD3 complex (the ζ chain) is undetectable (17). This mutant, which expresses ≈5% of wild-type levels of the other components of the receptor complex, retains signal transduction responsiveness to mAbs but not to specific antigen. It thus appears that the ζ chain is not essential to signal transduction by the murine antigen receptor but may play some role in modulating its expression on the cell surface or its sensitivity to various stimuli. Since both J.CaM1 and J.CaM2 express normal levels of receptor, it is unlikely that either is entirely lacking ζ chains, a fact that has been confirmed in immunoprecipitation studies and mRNA analysis (data not shown). However, in these cells undetected amino acid substitutions in any of the CD3 chains might cause more drastic interference in signaling by the receptor without affecting surface expression of CD3/Ti.

Since the predicted cytoplasmic domains of the Ti chains provide little apparent opportunity for intracellular communication, an associated "effector" subunit has been assumed to perform this function for the antigen receptor. The present mutants offer a hint that such components may exist and may provide reagents for their biochemical identification.

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