Previously we have described a system of somatic cell genetics (J.CaM1 and J.CaM2) for analyzing signal transduction via the T cell antigen receptor complex (CD3/Ti). Here we describe a third mutant, J.CaM3, which also expresses high levels of receptors that are functionally impaired. Like J.CaM1, J.CaM3 demonstrates partial signal transduction via CD3/Ti to only certain stimuli. J.CaM1, J.CaM2, and J.CaM3 define three non-Ti complementation groups involved in receptor function. To evaluate the mutations further we have introduced a heterologous receptor, the human muscarinic receptor 1 (HM1), into the parental Jurkat and mutant cell lines. This receptor demonstrates signal transduction competence in all these hosts, indicating that 1) T cells express the necessary apparatus for the coupling of HM1 to second messenger generation and 2) the mutations in the J.CaM family all affect molecules that are specific to CD3/Ti, and not HM1, function. Finally, the HM1 receptor exhibits partial sensitivity to cholera toxin in Jurkat cells, in contrast to the virtually complete sensitivity of CD3/Ti to cholera toxin.

The human T cell antigen receptor is among the large class of receptors that utilize the inositolphospholipid second messenger system to initiate cellular activation (1). Either the recognition of foreign antigens in the appropriate major histocompatibility context or the binding of monoclonal antibodies (mAbs)1 reactive with extracellular determinants of the receptor lead to the activation of intracellular phospholipase C (2). The resulting hydrolysis of phosphatidylinositol 4,5-bisphosphate generates cytosolic inositol 1,4,5-trisphosphate (IP3) and membrane-associated diacylglycerol, second messengers that promote elevation of intracellular free Ca2+ ([Ca2+]i) and activation of protein kinase C, respectively. The molecular means by which the antigen receptor communicates the ligand binding event to the intracellular compartment is largely unknown, although both conformational and receptor aggregation mechanisms have been proposed in other receptor systems.

The T cell antigen receptor is an elaborate multimolecular structure (2, 3). Gene transfer studies have demonstrated that the heterodimeric Ti subunit (consisting of the disulfide-linked α and β integral membrane glycoproteins) is responsible for recognition of antigen-major histocompatibility complex (4, 5). This function is consistent with the highly variable primary structure of the distal extracellular domains of the Ti chains. Still unknown is the precise function of the four or more invariant integral membrane proteins (the CD3 complex) (6) that appear to have obligate, noncovalent association with the Ti subunit (7, 8).

Because of the molecular complexity of the CD3/Ti complex, we have been utilizing a system of somatic cell genetics for studying structure/function relationships within the receptor-mediated signal transduction pathway. We have described two independently derived somatic cell mutants isolated from the Jurkat human leukemic T cell line (9, 10). While both express high levels of grossly normal antigen receptor complexes on the cell surface, they exhibit only partial (J.CaM1) or entirely absent (J.CaM2) coupling between CD3/Ti and phosphoinositide (PI) second messenger production. We recently demonstrated that both cell lines have non-Ti mutations that lie in different complementation groups, indicating that at least two non-antigen-binding molecules are critical to signal transduction by the receptor (10). The partial integrity of signaling function in response to some anti-CD3 (but not anti-Ti) mAbs in J.CaM1 led us to propose that CD3 normally is coupled directly to the second messenger system and that signal transduction involves transfer of information from Ti to CD3 and from CD3 to the next component in the pathway.

Of great interest in elaborating the molecular events underlying signal transduction by CD3/Ti is the identification of the mutant molecules in J.CaM1 and J.CaM2. Since a priori there are too many candidates to permit searching at the genetic level, one rational approach is to begin by exploring the functional consequences of the mutations on other receptor systems. For example, we found previously that a second T lineage-specific surface receptor (CD2) that is coupled to PI metabolism is also impaired in the J.CaM1 line, which had been selected specifically for defects in CD3/Ti function (11). These and other studies (12) suggested that CD2 function converges with that of CD3/Ti proximally in the signaling pathways. Since CD2 also depends functionally on surface expression of CD3/Ti, however, the relationship between these molecules appears to be complex. For further investigation of the pathways leading to second messenger production, we have now utilized a heterologous PI-coupled receptor (the human muscarinic subtype 1 receptor, HM1) for gene

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1 The abbreviations used are: mAb, monoclonal antibody; IP3, inositol 1,4,5-trisphosphate; PI, phosphoinositide; HM1, human muscarinic subtype 1 receptor; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; [3H]QNB, [3H]quinuclidinyl benzilate; EGTA, ethylenediaminetetraacetic acid; I, phosphorylcholine; CT, cholera toxin; IP4, inositol 1-phosphate; IP6, inositol 1-phosphate; CT, cholera toxin; IP4, inositol bisphosphate; IP, inositol phosphate.
transfer studies of the phenotypes of J.CaM1, J.CaM2, and a recently derived mutant, J.CaM3.

MATERIALS AND METHODS
Cell Lines—The human leukemic T cell line Jurkat (clone R6-1) and its derivative J.HT3-Ti3 were maintained in RPMI 1640 supplemented with 10% bovine foetal bovine serum (“medium”) as described (7). PEER (13) and MOLT-13 (14) were maintained similarly. J.CaM1 and J.CaM2 were isolated as described previously (9, 10). J.CaM3 was isolated as described for J.CaM1 but without lectin-mediated growth selection.

*Abs—*Abs C305 and R140 recognize idiotypes expressed on the Jurkat Ti (7, 15). WT31 recognizes a common Ti determinant (16). mAbs OKT3 (17), UCHT1 (18), A32.1 (19), anti-Leu4 (Becton Dickinson Monoclonal Center, Mountain View, CA), 235 (19), L1422, and L145 recognize CD3 epitopes.

Fluorometry, Fluorimetry, and Heterokaryon Assay—Cell surface immunofluorescence and flow cytometry were performed as described (7). The intracellular Ca++ concentration ([Ca++]i) was assessed by fluorimetry (1, 9) or by flow cytometry (9) with Indo-1 as described. Briefly, cells were incubated with the membrane-permeant pentakis(acetoxymethyl)ester (Molecular Probes, Junction City, OR) for 20 min at 37 °C (106 cells/ml, 3 μM Indo-1) followed by 10-fold dilution with medium and a second 20-min incubation. After extensive washing, continuous fluorescence determinations were made in a Fluorolog II spectrofluorimeter (Spex Industries, Edison, NJ) with excitation at 340 nm and emission detection at 400 nm. Levels of Indo-1 accumulated were compared with levels that had accumulated in the absence of agonist stimulation.

For analysis of heterokaryons (see below), multicolor immunofluorescence and flow cytometry were performed with a FACS IV (Becton Dickinson) with a 386-nm excitation beam for Indo-1 and a 501-nm excitation beam for fluorescein. Fluorescein emission was detected through a 515-nm band-pass filter (near the maximal emission for Ca++-bound Indo-1) or a 486-nm band-pass filter (near the maximal emission for Ca++-free Indo-1). Basal versus elevated [Ca++]i was determined by obtaining high levels of CD3/Ti on its surface, as demanded in the selection. Based on immunofluorescence and flow cytometry with the C305 (anti-Ti clonotypic), WT31 (anti-Ti framework determinant), and OKT3 (anti-CD3) mAbs, J.CaM3 expresses approximately 151 + 14% (S.D., n = 3) of the receptor levels found on the wild-type Jurkat cells. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of immunoprecipitated CD3/Ti proteins from J.CaM3 revealed the characteristic CD3 and Ti proteins that cannot be distinguished from those of Jurkat (data not shown). Two-dimensional isoelectric focusing analysis of immunoprecipitated proteins also revealed no gross differences in the CD3/Ti proteins (data not shown).

Despite the presence of high levels of receptor complexes recognized by available anti-CD3/Ti mAbs, J.CaM3 exhibited substantially impaired Ca++ mobilization responses to many of these mAbs. For example, J.CaM3 demonstrated virtually no elevation of [Ca++]i in response to the anti-Ti mAb R410 (Fig. 1B), which is a potent agonist for Jurkat cells (Fig. 1A). Similarly, J.CaM3 exhibited little response to the anti-CD3 mAb OKT3 (Fig. 1D) in contrast to the large response of Jurkat (Fig. 1C). However, addition of a cross-linking second antibody (i.e. rabbit anti-mouse Ig) appeared to reconstitute the agonist potential of OKT3 for J.CaM3 (Fig. 1D).

The responses of J.CaM3 to a large panel of mAbs are compared with those of Jurkat, J.CaM1, and J.CaM2 in Table I. This summary reveals that J.CaM3 is very similar to J.CaM1 in its responsiveness to certain anti-CD3 mAbs (235, anti-Leu4, L143, cross-linked OKT3, or C305 plus OKT3) and its lack of response to others (219, anti-Leu3, L30, and C305 alone). This pattern of unresponsiveness is consistent with the results of previous studies (7). J.CaM3 demonstrates virtual lack of expression of the OKT3 and C305 idiotypes as determined by flow cytometry. This was confirmed by immunoprecipitation analysis with [32P]orthophosphate and fluorescence autoradiography followed by autoradiography. Addition of cross-linking reagents (IgG or the cross-linking IgG fragment of goat anti-mouse Ig, not shown) reconstituted the agonist response of J.CaM3 to OKT3, as did addition of the soluble recombinant protein â3-chain fragment (not shown). Futhermore, this pattern of responsiveness was not due to the presence of high levels of receptor complexes recognized by available anti-CD3/Ti mAbs, J.CaM3 exhibited substantially impaired Ca++ mobilization responses to many of these mAbs.
of responsiveness to other anti-CD3 mAbs (OKT3, UCHT1, and A32.1) and to all anti-Ti mAbs examined (C305, R140, and WT31). Although the magnitude of these responses differ somewhat between the two mutants, the qualitative patterns of response are very similar. Therefore, unlike those of J.CaM2, the receptors of both J.CaM1 and J.CaM3 exhibit "leaky" defects in coupling to Ca2+ mobilization upon receptor engagement by mAbs.

Other receptor-mediated signaling events in J.CaM3 also appear to parallel the profile observed in J.CaM1. First, the production of inositol phosphate second messengers following ligand binding is significantly impaired. While Jurkat cells demonstrated a 501 ± 55% (S.E.) enhancement of IP3 production above basal levels in response to C305 (t = 10 min) and a 716 ± 44% increase in IP1 plus IP2 production, J.CaM3 cells demonstrated virtually no change in IP3 (110 ± 3%) or IP1 plus IP2 (82 ± 3%) levels, respectively. Therefore, as in J.CaM1 and J.CaM2, the signal transduction defect is proximal to IP second messenger production. Interestingly, as had been observed with J.CaM1, even the Ca2+-mobilizing mAb 235 caused only a small elevation of IP3 levels in J.CaM3 (161 ± 18%) compared with the wild-type cells (769 ± 64%) despite the "normal" immediate Ca2+ mobilization response. As we have suggested previously, therefore, the short-term mobilization of Ca2+ in these cells requires only a small elevation of IP3 (22).

Finally, our previously described heterokaryon complementation assay made possible the rapid assignment of J.CaM3 into a known or new complementation group (10). In this assay, partner cells are alternatively loaded with the Ca2+-sensitive fluorescent dye Indo-1 or stained with a fluorescein-tagged mAb reactive with an irrelevant surface marker, and the partners are then fused by brief exposure to polyethylene glycol. Within 1 h, the mixture of unfused parental cells and homo- and heterokaryons are assessed on the FACS IV by three-color analysis. The Indo-1-positive, fluorescein-positive heterokaryons formed by fusion between the mutant cell line (in this case, J.CaM3) and the test partner are assayed for Ca2+ mobilization responses to C305. As summarized (Fig. 2), J.CaM3 was complemented by Ti-β-deficient cells (J.RT3-T3.5 and MOLT13) and by Ti-α-deficient cells (PEER and MOLT13), but not by homokaryon (self) fusion. Therefore, as with J.CaM1 and J.CaM2, the Ti subunit is not the site of the defect in J.CaM3. Importantly, J.CaM3 was complemented by fusion with either J.CaM1 or J.CaM2, indicating that these three mutants all lie in different complementation groups (J.CaM1 and J.CaM2 were previously shown to complement one another (10)).

Introduction of HM1 Receptor into Jurkat Cells—The defects in the three J.CaM mutants all appear to reside proximal to the production of IP second messengers by CD3/Ti. By examining the influence of these mutations on non-CD3/Ti receptor function we hoped to acquire additional information about the normal contribution of the mutated molecules to signal transduction. The endogenous CD2 molecule exhibits a concomitant signaling defect in J.CaM1 (11), but CD2 also appears to depend on surface expression of CD3/Ti for signal transduction competence (11, 12). CD3/Ti function in Jurkat,

![Fig. 1. Calcium mobilization by the J.CaM3 and parental Jurkat cell lines.](image)

**TABLE I**

| Stimulus            | mAb      | Jurkat | J.CaM1 | J.CaM2 | J.CaM3 |
|---------------------|----------|--------|--------|--------|--------|
| Mean basal (S.D.)   |          |        |        |        |        |
| Anti-Ti             |          |        |        |        |        |
| C305                | 109 (30) | 94 (24)| 96 (10)| 110 (27)|
| WT31                | 1923     | 82     | 96     | 158    |
| R140                | 657      | 106    | ND*    | 299    |
| Anti-CD3            |          |        |        |        |        |
| OKT3                | 976      | 70     | 108    | 135    |
| UCHT1               | 336      | 80     | ND     | 187    |
| A32.1               | 1117     | 86     | 112    | 172    |
| Anti-Leu4           | 1322     | 243    | 107    | 477    |
| 235                 | 831      | 710    | 92     | 2575   |
| L142                | 1634     | 971    | 91     | ND     |
| L143                | 1119     | 875    | 104    | 1174   |
| Cross-linked        |          |        |        |        |        |
| OKT3                | ND       | 558    | 117    | 615    |
| Combination         | C305 + OKT3| ND    | 1209   | 108    | 506    |

* ND, not determined.
however, does not depend on the presence of CD2 (23). Since both J.CaM2 and J.CaM3 have spontaneously lost expression of CD2, it would be difficult to assess CD2 function in these cells. Instead, we chose to introduce a heterologous PI-coupled receptor into the parental and mutant lines and to evaluate its signaling function in these hosts.

The human muscarinic acetylcholine receptor subtype 1 is a PI-coupled member of the seven-transmembrane domain receptor family (24). For expression in the Jurkat family of cells, an expression plasmid was constructed by replacing the Ti-β cDNA in the pTβFNeo vector (8) with the genomic HM1 clone (kindly provided by D. Capon and E. Peralta, Genentech). In the resulting plasmid (Fig. 3A, pHM1-SFNeo) the HM1 gene is driven by the Friend spleen focus-forming virus enhancer sequence.

pHM1-SFNeo was introduced into Jurkat by electroporation and stable transfectants were selected in G418, as previously described (10). Individual clones were screened for receptor expression by assessing saturation isotherm binding of the muscarinic receptor antagonist [3H]QNB. While parental Jurkat cells demonstrated virtually no detectable [3H]QNB binding (data not shown), the representative clone J-HM1-2.2 demonstrated significant saturable binding ("total"") in Fig. 3B). Binding of [3H]QNB to J-HM1-2.2 was virtually completely inhabitable by atropine, a second muscarinic-specific antagonist ("nonspecific") in Fig. 3B). The binding kinetics from triplicate reactions for each concentration were analyzed using the microcomputer-based LUNDON-1 saturation isotherm data analysis program (the Edie-Hofstee one-site binding model). These analyses demonstrated that J-HM1-2.2 exhibits approximately 9000 binding sites per cell, with a Kd for [3H]QNB binding of 32 pm, which is consistent with published reports of 17-58 pm. The calculated number of HM1 receptors is similar to the number of antigen receptors expressed on Jurkat cells.3 Northern blot analysis also confirmed the presence of HM1 transcripts in transfected cells but not in native Jurkat cells (data not shown).

**Signaling Function of HM1 in Jurkat and CD3/Ti-negative Variant** —Since the HM1 receptor has been shown to exhibit coupling to the PI second messenger system in other hosts (24) we examined this function in the Jurkat transfectants. In Jurkat a very sensitive indicator of IP3 production is an elevation of [Ca2+]. In Ca2+ fluorimetry studies untransfected Jurkat cells demonstrated no change in [Ca2+], in response to the muscarinic agonist carbamylcholine (carbachol), but not in native Jurkat cells (data not shown).

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3 M. Graber, personal communication.
for HM1 expression, a positive clone was found (J-HM1-2.1) that had spontaneously lost expression of CD3/Ti from the cell surface. The absence of CD3/Ti was demonstrated both by immunofluorescence assays which revealed no detectable CD3/Ti. J-HM1-2.1 was a HM1-CD3/Ti- cell line. J-HM1-2.2 is a HM1-CD3/Ti+ cell line. Carbachol (Carb., final concentration 100 μM), atropine (final concentration 10 μM), and C305 or OKT3 (ascites, final concentration 1:1000 dilution) were added where indicated.

Fig. 5. Production of inositol phosphates by Jurkat and Jurkat-derived HM1 transfectant (J-HM1-2.2). Inositol phosphates were extracted and analyzed as described under "Materials and Methods."" With the injection of 10 μM LiCl was assessed following 20 min of exposure to either carbachol or C305. The saturating and subsaturating doses were chosen from dose-response curves (not shown) which indicated the maximal and approximate EC_{50} for each ligand. Shown are mean ± standard error of the mean (n = 3).

Table II

| Stimulus       | IP production | Inhibition* |
|----------------|---------------|-------------|
|                | Without CT    | With CT     | % inhibition |
| Carbachol      | 985 ± 21      | 412 ± 14    | 65           |
| Saturating     | 759 ± 4       | 325 ± 5     | 66           |
| Subsaturating  | 703 ± 14      | 142 ± 5     | 93           |
| C305           | 445 ± 10      | 111 ± 8     | 97           |

*Calculated as % inhibition of induced production.

Fig. 6. Production of inositol phosphates in HM1 transfectants of Jurkat, J.CaM1, J.CaM2, and J.CaM3. A total combined inositol phosphate fraction was eluted and counted. Stimulations (in the presence of 10 mM LiCl) were with nothing ("unstimulated"), carbachol (100 μM), or C305 (ascites, final dilution 1:1000) for 20 min. Error bars indicate standard errors of the mean (n = 3).
Heterologous Receptor Function in Somatic Cell Mutants

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ment (Table II), with only 65–66% inhibition of the induction of IP production at saturating or subsaturating doses of carbachol. The virtually complete inhibition of CD3/Ti function and the limited inhibition of HM1 function was a consistent finding in multiple experiments. Similar 3-h incubations with the AMP analogue dibutyryl-cAMP at doses from 1 to 500 μM caused no inhibition of the IP responses of J-HM1-2.2 to either anti-Ti or HM1 agonists (data not shown).

HM1 Function in CD3/Ti Signaling Mutants—To aid in defining the mutated molecules in the signaling mutants J.CaM1, J.CaM2, and J.CaM3 were transfected with pHM1-SFNeo, and receptor-bearing clones were isolated as described for Jurkat. Northern analysis demonstrated significant HM1 expression in three representative transfectants derived from these mutants (data not shown), although the transfected J.CaM3 cells consistently expressed lower levels than did the other mutants. The number of HM1 receptors per cell was assessed by binding isotherm analysis with [3H]QNB, yielding J.CaM1-HM1-1.3 = 14,000 sites/cell, J.CaM2-HM1-1.4 = 12,000 sites/cell, and J.CaM3-HM1-1A.12 = 2,000 sites/cell.

The functional capacity of the transfected HM1 receptor was assessed by IP production, since early Ca2+ mobilization is not directly proportional to IP3 production. While endogenous CD3/Ti in all the transfected mutants failed to mediate IP production, as expected, the HM1 receptors were competent in the context of all three CD3/Ti signaling mutants (Fig. 6). Carbachol elicited 7–8-fold increases in total IP production in transfected J.CaM1 and J.CaM2, and 3.5-fold increases in J.CaM3; the lower response in J.CaM3 probably is attributable to the lower receptor number, since the transfected wild-type Jurkat cells also demonstrated a quantitative dependence on receptor number (data not shown). Ca2+ mobilization responses to carbachol were similar in both transfected mutants and transfected wild-type cells, and the relative production of individual IP metabolites were comparable in the mutant and wild-type hosts (data not shown). Therefore, the mutations in the J.CaM family all affect molecules that function in an antigen receptor-specific signal transduction system and therefore have no effect on the HM1 signal transduction function.

DISCUSSION

The molecular complexity of the T cell antigen receptor complex and the lack of a cell-free reconstitution system prompted us and others to employ somatic cell genetics to explore structure/function relationships within this receptor system. Previous studies of the mutants J.CaM1 and J.CaM2 demonstrated that each has a different signaling deficit, with J.CaM1 exhibiting partial responsiveness to some anti-CD3/Ti mAbs and J.CaM2 exhibiting a fully nonresponsive phenotype to all mAbs assessed (9, 10, 15). Complementation studies involving somatic cell hybridization, gene transfer, and a heterokaryon assay indicated that the Ti subunit is normal in both mutants (10). Moreover, J.CaM1 and J.CaM2 complement each other, suggesting that each cell line has a mutation in a different non-antigen-binding molecule involved in the CD3/Ti signal transduction process (10). We proposed that the partial integrity of responses of J.CaM1 to certain anti-CD3 mAbs and the absence of Ti-mediated signaling function implies that CD3 itself may be serve to couple Ti to other components of the signal transduction pathway.

The present studies of the new mutant J.CaM3 extend the genetic analysis of T cell antigen receptor function. Like the earlier mutants, J.CaM3 expresses high levels of grossly normal CD3/Ti complexes. Both electrophoretic analysis of immunoprecipitated Ti and CD3 proteins and epitope studies using immunofluorescence with anti-CD3/Ti mAbs offer no clues as to the structural basis of impaired receptor function. Despite an abundant levels of receptor on the cell surface, however, J.CaM3 exhibits impaired signal transduction like that of J.CaM1. First, only certain anti-CD3 mAbs elicit mobilization of intracellular Ca2+ while anti-Ti mAbs and other anti-CD3 mAbs are less effective. These features imply that the defect in signaling is relatively proximal in the pathway. Second, the failure to promote Ca2+ fluxes is attributable to the absence of coupling to IP production, also implying an “upstream” mutation. Third, those mAbs that do retain agonist function (e.g. 235) for J.CaM3 and for J.CaM1 in terms of Ca2+ mobilization appear to have only minimal agonist function with regard to IP production in the two cell lines.

The similarity of phenotypes between J.CaM1 and J.CaM3 is particularly intriguing in view of the complementation studies demonstrating that the mutations in the two cell lines lie in different complementation groups, both of which are distinct from the complementation groups defined by Ti. These findings suggest that the mutations in J.CaM1 and in J.CaM3 affect different molecules in the signaling apparatus, implying the necessity of a third non-antigen-binding molecule in the coupling of the receptor to second messenger production. While little information is available regarding the identity of the mutated molecule in J.CaM2, the “leaky” phenotypes of J.CaM1 and J.CaM3 are suggestive of early components in the pathway. The existence of mutations affecting two separate components but causing nearly indistinguishable partially permissive phenotypes is consistent with the possibility of a multimolecular complex subserving signal transduction function for the receptor. In such a model, two proteins with related functions might both be anticipated to be potential loci for mutations leading to similar functional deficits, as seen with J.CaM1 and J.CaM3. It is tempting to hypothesize that the well characterized CD3 complex represents the functional complex implicated by the genetic analyses, and that J.CaM1 and J.CaM3 will be found to have mutations in different but related CD3 chains or associated proteins. For example, in the murine T cell system there is some evidence linking expression of the CD3-γ/δ dimer to the signal transduction competence of the receptor complex (30). Molecular analyses are currently in progress to test this hypothesis.

As a further tool in dissecting the signal transduction pathways, we have utilized gene transfer to introduce a heterologous PI-coupled receptor into the Jurkat-derived cell lines. Previous studies of the endogenous T cell-specific receptor CD2 demonstrated that the function of this molecule is dependent on both the expression and functional integrity of the CD3/Ti complex (11, 12). Expression of the HM1 receptor in Jurkat-derived cells allowed us to explore the relationship of this receptor with that of the CD3/Ti complex. Although native Jurkat cells express subdetectable HM1 receptors, the present studies indicate the availability of the appropriate machinery for HM1 function. Levels of HM1 receptor in transfected cells that are similar to those of endogenous CD3/Ti complexes mediate comparable induction of second messenger production upon exposure to receptor agonist; the acetylcholine analogue carbachol promotes both production of IP3 and elevation of [Ca2+]i, as do CD3/Ti ligands. Receptor-mediated activation of second messenger production is completely and immediately reversible by atropine, a muscarinic antagonist. Furthermore, the coupling of HM1 to second messenger production in T cells does not depend on the expression of functional CD3/Ti complexes, suggesting that such dependence is a unique property of T lineage-specific
receptors such as CD2 (11, 12), Thy-1 (31), Tp103 (32), and Ly5 (33).

To characterize these signaling pathways, we examined the influence of cholera toxin on HM1 and CD3/Ti function. Others have demonstrated that prolonged exposure of Jurkat cells to CT virtually completely inhibited CD3/Ti signaling function independently of elevations of cAMP (28). While the mechanism of inhibition of CD3/Ti function is unknown, it is possible that an characterized G protein that mediates antigen receptor signaling is a substrate for CT, resulting in its debilitation. Other possible mechanisms for this inhibition include indirect effects which lead to receptor or coupling protein modification, or inositol phospholipid depletion (34). In the present studies we confirmed the complete inhibition of CD3/Ti function and observed only a partial inhibition of HM1 function. The partial impairment of HM1 function contrasts with previous studies showing the absence of CT and pertussis toxin effects in native hosts. Neither receptor system appeared to be affected by even high concentrations (500 μM) of dibutyryl-cAMP, suggesting that the CT effects on CD3/Ti and HM1 may not be mediated by the concomitant elevation of cAMP caused by G, activation. This is in contrast to some studies in which cAMP or its analogues have been shown to inhibit signal transduction by the T cell receptor in a murine T cell hybridoma (35). In Jurkat cells, however, the effects of cholera toxin cannot be attributed solely to the elevated levels of cAMP that are induced (25, 36). Definitive studies to resolve these questions may require more extensive somatic cell genetics involving the cAMP-mediated kinase pathways.

The differential sensitivity of the CD3/Ti and HM1 receptor systems suggests two conclusions. First, CT-mediated inhibition of receptor function in these cells is unlikely to result from depletion of inositol phospholipid substrates or impairment of phospholipase C, since such mechanisms might be expected to influence equivalently all receptor-mediated signaling processes that utilize this second messenger system. Second, the HM1 and CD3/Ti systems both appear to involve CT-sensitive components, but these components must not be wholly identical in view of the quantitatively different effects. Whether or not the relevant CT substrates are G proteins remains to be determined. Recent work with the muscarinic receptor expressed in heterologous hosts (Chinese hamster ovary cells) suggested strongly that a cell can express multiple distinct G proteins capable of coupling to phospholipase C and that a given receptor type can utilize more than one variety of G protein in such an environment (26). Therefore, potential explanations for the partial sensitivity of HM1 to CT in Jurkat cells include: (a) use of a G protein by HM1 that is distinct from, but related to, that of CD3/Ti and that is partially CT-sensitive; and (b) use of two G proteins by HM1, one of which is completely resistant to CT and one of which is sensitive to it (i.e., the putative CD3/Ti G protein). Further studies with cAMP analogues and with cAMP-dependent protein kinase mutants should help to clarify some of these important issues.

The introduction of a heterologous receptor into the J.CaM family of mutants provided an additional means to dissect the various components and pathways leading to intracellular second messenger generation. Since the mutants define a minimum of three non-Ti components of the antigen receptor signaling system, we predicted that some of the mutations might affect segments of the system that are common to other receptor-mediated signaling pathways. Based on previous work with CD2 we hypothesized that there might be a general interdependence among cell surface receptors. The experiences with the transfected mutants clearly demonstrated, however, that the HM1 receptor pathway is largely independent of the CD3/Ti pathway. None of the three mutants demonstrated substantially impaired signal transduction via the heterologous HM1 receptor, despite their deficiencies in CD3/Ti function. These findings imply that the mutations in these cells affect components that are used specifically by the antigen receptor, a conclusion that is consistent with the hypothesized antigen receptor-associated multimolecular signal transduction apparatus that may be altered in the mutants.

The mutant and heterologous receptor model systems should permit further exploration of the molecular interactions underlying the signal transduction function of the antigen receptor. It should also be possible to make direct comparisons of the abilities of the antigen and muscarinic receptors to mediate the necessary signals for cellular activation. Finally, molecular analysis of the known receptor components may provide insight as to the loci of the mutations in the J.CaM family and as to the role of the affected molecules in receptor function.

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