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# REQUIREMENT FOR THE COEXPRESSION OF T3 AND THE T CELL ANTIGEN RECEPTOR ON A MALIGNANT HUMAN T CELL LINE

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During the past year, several laboratories have developed monoclonal antibodies reactive with idiotypic-like determinants expressed on human and murine T cell clones, lines, or hybridomas (1-5). These antibodies are felt to react with the antigen receptors of these cells. Such antibodies can mimic the effects of antigen by specifically activating the immunizing T cell, or, under appropriate circumstances, can specifically block the antigen reactivity of the immunizing T cell (2-7). Immunoprecipitates prepared from different cells with such antibodies contain distinct heterodimers of 80-90 kilodaltons (kD) under nonreducing conditions and two chains of 37-52 kD under reducing conditions (1-7).

On human cells, another set of molecules, T3, has been associated with such putative T cell antigen receptors. T3 consists of at least three noncovalently linked distinct peptides, each of which has not been shown to exhibit any structural or antigenic polymorphism (8). Several observations have suggested an association between T3 and the putative antigen receptor: T3 co-modulates with antigen receptor determinants on T cell clones (2); the induction of an unresponsive state in T cell clones exposed to soluble antigen is accompanied by a diminished expression of T3 (9); T3 and receptor binding sites are stoichiometric (10); immunoprecipitates of T3, under some conditions, contain the T cell receptor heterodimer in addition to the peptides representing T3 (11); and the appearance of T3 during T cell ontogeny has been linked to the expression of antigen receptor-like molecules on T cells (12). Despite these observations, however, the role of T3 in this association has not been defined.

T3 may play a critical role in T cell activation. Monoclonal antibodies reactive with T3 can provide one of the two requisite signals required for T cell activation (13-15). Thus, such antibodies can induce T cell activation in a manner analogous to that noted with antigen or anti-receptor antibodies. This transmembrane activation signal is mediated by an increase in cytoplasmic free calcium (16). The studies reported here examined the association between T3 and antigen receptor-like molecules expressed on a functional human T cell line, Jurkat, using mutants of Jurkat. These studies demonstrate that there appears to be an obligate requirement for the coexpression of T3 and the antigen receptor. Moreover,

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the expression of these molecules appears to be required for the activation of Jurkat cells by the lectin phytohemagglutinin (PHA).<sup>1</sup>

### Materials and Methods

**Cells.** An interleukin 2 (IL-2)-producing clone of Jurkat, E6-1, and mutants of this clone (described below) were passaged in RPMI 1640 supplemented with 10% fetal bovine serum as previously described (15). CTLL-20, a mouse IL-2-dependent T cell line, was passaged in IL-2-supplemented medium as previously described (15).

**Monoclonal Antibodies.** A monoclonal antibody with specificity for an antigen receptor-like molecule expressed on Jurkat was derived from a fusion between SP-2/0 mouse myeloma cells and spleen cells from BALB/c mice that had been immunized intraperitoneally with  $5 \times 10^6$  Jurkat cells every month for 3 mo. The fusion was performed according to previously described methods 3 d after an intravenous immunization with the same number of Jurkat cells (17). One hybrid, C305, proved to be specifically reactive with Jurkat. Culture supernatant fluid or ascitic fluid from this hybridoma was used for the studies described in this report. Another antibody derived from this fusion, C373, proved to be reactive with the proteins associated with the E rosette receptor on human T cells, based on its T cell specificity, ability to inhibit E rosette formation, and immunoprecipitation of a 55 kD peptide on T cells. Both C305 and C373 are IgM kappa immunoglobulins. OKT3, anti-Leu-4, 64.1, anti-Leu-1, anti-Leu-5, W6/32, MOPC-195, and MOPC-104E (IgM) monoclonal antibodies were obtained from previously described sources (15).

**Preparation of Radioiodinated Cell Surface Proteins.** Iodination of cell surface molecules was performed using glucose oxidase catalyzed of the lactoperoxidase technique (18). Cells were lysed at  $4.0 \times 10^7$ /ml in ice-cold lysis buffer containing 1.0% Nonidet-40 (Particle Data, Inc., Elmhurst, IL), phenylmethylsulfonyl fluoride (1 mM) and benzamide (1 mM) in 0.01 M Tris-HCl, pH 8.0, and iodoacetamide, 10 mM, in 0.15 M NaCl (lysis buffer). After 30 min of incubation at 4°C, nuclei and large debris were removed by centrifugation. Lysates were then precleared with formalin-fixed *Staphylococcus aureus* (Calbiochem-Behring Corp., La Jolla, CA), centrifuged at 100,000 g for 30 min, and supernatants were stored at -70°C.

**Characterization of Radioiodinated Cell Surface Proteins.** Cell surface molecules were characterized using the immunoisolation technique described by Tamura et al. (19). One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of isolated cell surface proteins was performed on 10% acrylamide slab gels with a discontinuous buffer system according to previously described methods (20). The molecular weight standards (Pharmacia Fine Chemicals, Piscataway, NJ) run in parallel lanes were visualized using Coomassie Blue staining.

Diagonal, two-dimensional SDS-PAGE was performed on whole cell lysates or isolated proteins under nonreducing conditions in the first dimension using tubes containing a 0.2 × 10-cm 10% gel with a 0.2 × 1-cm 3% stacking gel. Gels were then equilibrated in SDS sample buffer containing 5% 2-mercaptoethanol for 30 min at 22°C and the second dimension electrophoresis was performed using a 10% acrylamide slab gel. Autoradiographs of the dried gels were visualized using intensifying screens and Kodak XAR-5 film.

**Derivation of Jurkat Mutants.** Mutants of Jurkat cells were produced by exposing cells to ethyl methane sulfonate (EMS) (Sigma Chemical Co., St. Louis, MO), 200 µg/ml, for 24 h or to 200 rad of gamma radiation (cesium source). After an additional 5 d of culture,  $1-2 \times 10^6$  mutagenized cells were negatively selected with OKT3 (1:100 final dilution) or C305 ascitic fluid (1:100 final dilution) and rabbit serum (1:8 final dilution) as a source of complement for 60 min at 37°C. This negative selection was performed at least three

<sup>1</sup> *Abbreviations used in this paper:* EMS, ethyl methane sulfonate; IL-2, interleukin 2; MCF, mean channel fluorescence; MHC, major histocompatibility complex; PBL, peripheral blood monocytes; PHA, phytohemagglutinin; PMA, phorbol myristate acetate; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS-polyacrylamide electrophoresis.

times over a 2 wk period. Positive selection of T3- or C305-negative cells was performed using indirect immunofluorescence and sorting negative cells by flow cytometry as previously described (16). Positive selection was always performed by staining the treated cells with the same antibody used in the negative selection treatment. Sorted negative cells were cloned by limiting dilution.

*Indirect Immunofluorescence, Flow Cytometric Analysis, and Sorting.* Cells were stained with hybridoma supernatants (undiluted) or commercial antibodies followed by fluoresceinated F(ab')<sub>2</sub> goat anti-mouse Ig (Cappel Laboratories, Cochranville, PA) as previously described (15). Cells were analyzed and sorted sterilely on a FACS IV fluorescence-activated cell sorter (Becton, Dickinson & Co, Sunnyvale, CA) according to previously described methods (16).

*Quantitative Absorption Studies.* Serial twofold dilutions of cells used for absorptions were incubated in 100  $\mu$ l of OKT3 (1:800) or C305 ascitic fluid (1:1000) for 30 min at 22°C. Supernatants were collected and 50  $\mu$ l were added to 25  $\mu$ l of medium containing 10<sup>4</sup> Jurkat cells labeled with <sup>51</sup>Cr according to previously described methods (17). To this, 25  $\mu$ l of a 1:8 dilution of rabbit serum that had been preabsorbed with Jurkat cells was added. Cells were incubated for 60 min at 37°C and the percent specific <sup>51</sup>Cr release was determined as previously described (17).

*Stimulation of Jurkat and Mutants and Determination of IL-2 Activity.* Jurkat or mutant cells were stimulated at 1  $\times$  10<sup>6</sup> cells/ml in microtiter wells as previously described (15). The final concentration of the stimulating agents was: PHA (Burroughs Wellcome Co., Research Triangle Park, NC), 1  $\mu$ g/ml; phorbol myristate acetate (PMA) (Sigma Chemical Co.), 50 ng/ml; commercial monoclonal antibodies, 1:100 dilution; C305 or C373 hybridoma culture supernatant, 1:20–1:100 dilution; and A23187 (Calbiochem-Behring Corp.), 1  $\mu$ g/ml. After 24 h of culture, cell-free supernatants were assessed for IL-2 activity using the IL-2-dependent line, CTLL-20, according to previously described methods (15). The lower limit of detection in this assay is 2.0 U/ml.

## Results

*Characterization of the Molecules Recognized by Monoclonal Antibody C305.* To study the association between T3 and the antigen receptor, a monoclonal antibody (C305) reactive with a putative antigen receptor expressed on the T3-positive human T cell line, Jurkat, was produced. C305 reacted with Jurkat but failed to react with B cell lines, peripheral blood lymphocytes (PBL), or other T cell lines as assessed by indirect immunofluorescence or antibody and complement-mediated cytotoxicity. Moreover, C305 failed to react with two other available T3-positive human T cell lines, HUT 78 and PEER. Fig. 1 depicts the flow cytometer fluorescent histograms obtained with Jurkat, PBL, and HUT 78 stained with OKT3 (reactive with T3), C305, and anti-Leu-1 (reactive with T1). As is evident, C305 appears to react with an antigenic determinant uniquely expressed on Jurkat. This is contrasted by the histograms obtained with OKT3 or anti-Leu-1 which reacted with 95% of Jurkat or HUT 78 cells as well as with the T cells within the PBL population.

The nature of the molecules bearing determinants with which C305 reacted were defined by isolation of radioiodinated Jurkat cell surface proteins. By one-dimensional SDS-PAGE, the protein with which C305 reacted migrated as a diffuse band of 80–90 kD under nonreducing conditions (Fig. 2F). Under reducing conditions, this protein could be resolved into two major peptides of 42 and 54 kD (Fig. 2E). In some studies performed under reducing conditions (see below), a third band of intermediate molecular mass has been visualized, perhaps representing a degradation product or an incompletely glycosylated

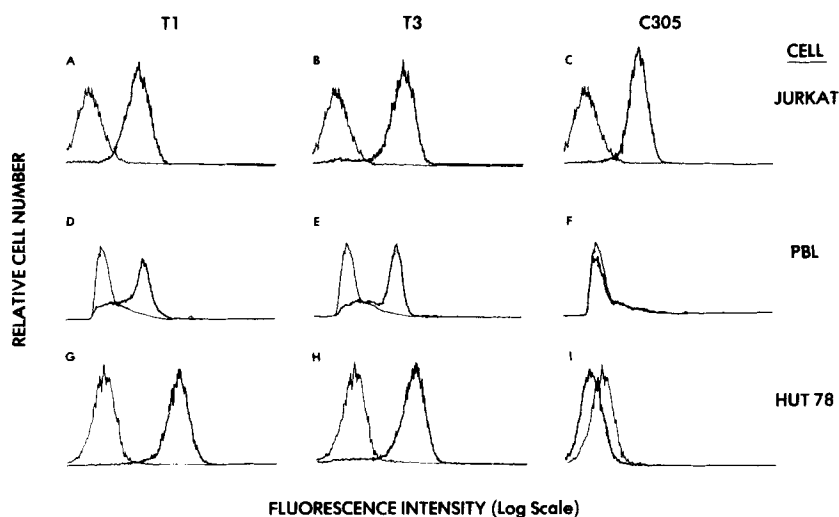


FIGURE 1. C305 reacts with idiotypic-like determinants expressed on Jurkat. Shown are fluorescence histograms obtained on a flow cytometer of Jurkat cells (A-C), PBL (D-F), and HUT 78 cells (G-I) stained by indirect immunofluorescence with antibodies of the indicated specificities (heavy lines). In each case, the histogram obtained with a nonreactive control antibody (light line) is shown for comparison.

form of the 54 kD band. Similar observations have been made in the murine system (5). Thus, this heterodimer isolated from Jurkat is very similar in molecular mass to the putative T cell receptors isolated by others from human T cell clones and lines (1-5). C305 reacts with a molecule distinctly different from T3 which is represented by the broad band of 22-24 kD and the band of 28 kD isolated from Jurkat using an anti-T3 monoclonal antibody (Fig. 2B). These results, together with the cell specificity data, suggest that C305 reacts with idiotypic-like determinants expressed on a heterodimer with structural characteristics of the putative T cell antigen receptor.

*C305 Can Activate Jurkat Cells.* Previous studies demonstrated that Jurkat cells could be activated to produce IL-2 by PHA (15, 16, 21). This response was greatly augmented by the phorbol ester, PMA. Anti-T3 monoclonal antibodies could activate Jurkat to produce IL-2, but only if PMA was also added (Table I; 0 vs. 109 U/ml of IL-2) (15). In agreement with previous results from this laboratory (15), monoclonal antibodies reactive with T1, T11, or HLA determinants failed to activate Jurkat even in the presence of PMA (Table I). A monoclonal antibody reactive with an antigen receptor on a responsive cell might be expected to function in a manner analogous to antigen by its ability to activate the cell. Jurkat cells could, indeed, be activated by C305 if PMA was added (Table I). The magnitude of the response to C305 plus PMA was comparable to that obtained with OKT3 plus PMA (119 vs. 109 U/ml, respectively). Thus, the clonotypic antibody C305, which has specificity for an 80-90 kD heterodimer, could activate Jurkat, supporting the notion that C305 reacts with the antigen receptor expressed on Jurkat.

*T3-negative Mutants of Jurkat Fail to Express the Idiotypic Determinants Recognized by C305.* In a previous study (16), a T3-negative mutant of Jurkat produced in

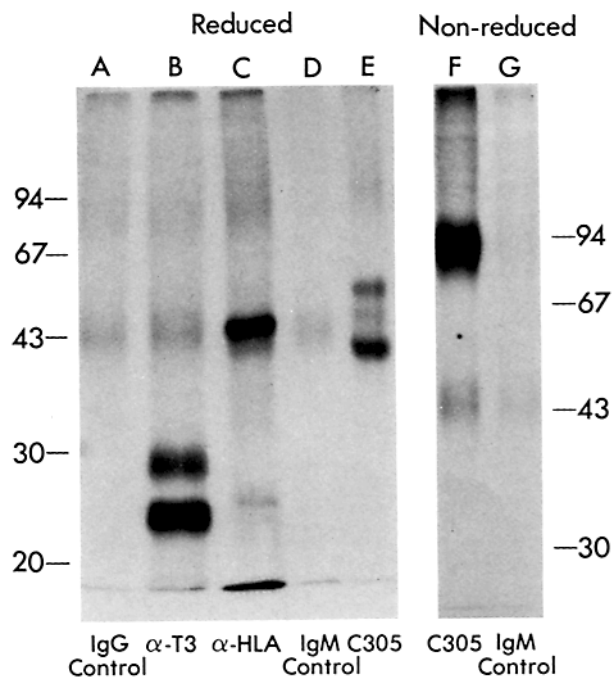


FIGURE 2. C305 reacts with a heterodimer expressed on the surface of Jurkat cells. Cell surface molecules of Jurkat were iodinated, solubilized in Nonidet P-40, isolated, and electrophoresed by SDS-PAGE as described in Materials and Methods. Molecules in lanes A-E and F-G were isolated and electrophoresed under reducing and nonreducing conditions, respectively. Antibodies used for the isolation were as follows: (A) MOPC-195 (IgG), nonreactive with Jurkat; (B) anti-Leu-4, reactive with T3; (C) W6/32, reactive with HLA; (D and G) 104E (IgM), nonreactive with Jurkat; (E and F) C305, reactive with the putative antigen receptor expressed on Jurkat.

this laboratory was described. This mutant, S.5, was produced by mutagenization with EMS followed by negative selection with OKT3 and complement and subsequent positive selection of cells lacking T3 by flow cytometry. Since the expression of T3 has been associated with the antigen receptor on T cell clones, it was of interest to examine S.5 for the expression of the determinants recognized by C305.

Initial studies suggested that, indeed, S.5 failed to express antigenic determinants recognized by C305. This was more rigorously assessed in quantitative absorption studies. Whereas the wild-type, T3-bearing Jurkat cells absorbed anti-T3 antibody activity in a dose-dependent fashion, neither S.5 nor the B cell line were able to absorb the anti-T3 activity (Fig. 3A). From these results it can be deduced that S.5 and the B cell line express  $<1/64$ th the quantity of T3 that is expressed on Jurkat. Similarly, although Jurkat cells were readily able to absorb C305 activity, both S.5 and the B cell line failed to absorb C305 (Fig. 3B). These results suggest that S.5 not only failed to express detectable T3 determinants but also lacked detectable determinants recognized by C305.

In further studies, depicted in Fig. 4, the specificity of the lack of expression of T3 and determinants recognized by C305 on S.5 was examined by indirect immunofluorescence as assessed by flow cytometry. The histograms obtained

TABLE I  
C305 Can Activate Jurkat

Antibody	Specificity	Isotype	IL-2 (U/ml)	
			-PMA	+PMA
OKT3	T3	IgG 2	0	109 ± 30.0
C305	Antigen receptor	IgM	0	119 ± 11.1
Anti-Leu-1	T1	IgG 2	0	0
Anti-Leu-5	T11	IgG 1	0	0
C373	T11	IgM	0	0
W6/32	HLA	IgG 2	0	0

Jurkat cells,  $1 \times 10^6$ /ml, were stimulated with culture supernatants of C305 or C373 (1:20 final dilution) or commercial antibodies (1:100 final dilution) in the absence or presence of PMA (50 ng/ml) for 24 h. Resultant IL-2 activity in the culture supernatants were assessed as described. Results represent the mean  $\pm$  SEM of three experiments and are expressed as units of IL-2 per milliliter.

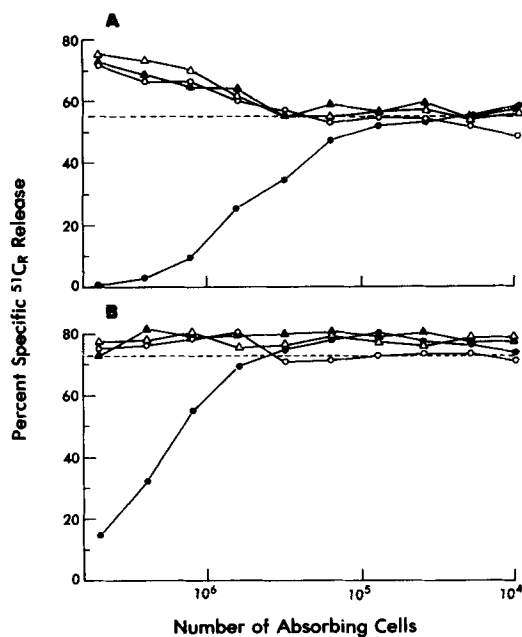


FIGURE 3. T3 antigenic determinants and determinants recognized by C305 are lost concomitantly on mutants of Jurkat as assessed by quantitative absorption. 100  $\mu$ l of a 1:800 dilution of OKT3 (A) or a 1:1,000 of C305 (B) were absorbed on decreasing numbers of Jurkat (●), S.5 (○), J.RT-C305.6 (▲), or an EBV-transformed B cell line (Δ). Residual antibody activity in the absorbed supernatants was assessed in an antibody plus complement-mediated assay using <sup>51</sup>Cr-labeled Jurkat cells as described in Materials and Methods. The dashed lines represent the cytolytic activities of the unabsorbed antibodies.

with S.5 cells stained with anti-T3 (Fig. 4F) or C305 (Fig. 4G) monoclonal antibodies were not appreciably different than those obtained with the negative control antibodies. The fluorescent histograms obtained when S.5 was stained with anti-Leu-1 (Fig. 4H, anti-T1), anti-Leu-5 (Fig. 4I, anti-T11), and anti-HLA (Fig. 4J) demonstrate that S.5, like the wild-type Jurkat cells, express determinants detected by these antibodies. The inability to detect T3 determinants on

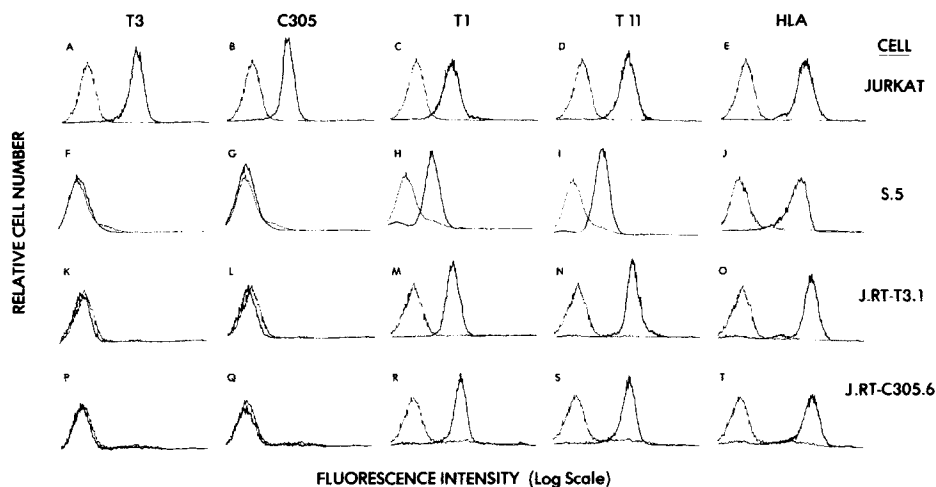


FIGURE 4. Specific loss of T3 antigenic determinants and determinants recognized by C305 on mutants of Jurkat. Jurkat (A-E), S.5 (F-J), J.RT-T3.1 (K-O), and J.RT-C305.6 (P-T) were assessed for their expression of the indicated antigenic specificities by indirect immunofluorescence and flow cytometric analysis (heavy lines). In each case, the histogram obtained with a nonreactive control antibody (light line) is shown for comparison.

S.5 by indirect immunofluorescence was confirmed in previous studies with two additional independently derived anti-T3 antibodies, although the chain(s) with which these antibodies react has not been firmly established (16).

The above results suggested that there might be an obligate requirement for the coexpression of T3 and the antigen receptor. To further examine this notion, Jurkat cells were independently mutagenized using either EMS or  $\gamma$  radiation in three separate experiments. An aliquot of cells was then treated with either anti-T3 plus complement or C305 plus complement. Anti-T3-treated cells were then selected for T3 negativity and C305-treated cells for C305 negativity on a fluorescence-activated cell sorter. Sorted cells were then cloned by limiting dilution and screened for the absence of determinants recognized by the antibody used in the selection procedure. In four independent mutagenizations, regardless of the mutagen, or the antibody used in the selection, every negative clone analyzed ( $n = 30$ ) failed to express either T3 or the antigenic determinants recognized by C305 (summarized in Table II). For example, J.RT-C305.6, a radiation-induced mutant selected for its failure to react with C305, failed to express molecules detected by anti-T3 monoclonal antibodies. The failure to detect antigenic determinants of T3 or those recognized by C305 on J.RT-C305.6 was confirmed by quantitative absorption (Fig. 3), indirect immunofluorescence (Fig. 4), and antibody plus complement-mediated cytotoxicity (data not shown). Similar results were obtained with the other negative clones listed in Table II. These results provide further support for the notion that T3 and the antigen receptor are associated on the plasma membrane. More importantly, they suggest an obligate requirement for the coexpression of T3 and the antigen receptor.

In the course of screening for T3-negative mutants, several clones were detected that expressed a markedly diminished but not absent level of T3. One



TABLE II  
*Mutants of Jurkat Demonstrate an Obligate Requirement for the Coexpression of T3 and the Antigen Receptor*

Experiment	Cell	Mutagen	Antibody Selection	Relative antigen expression*				
				T3	C305	T1	T11	HLA
1	S.5	EMS	OKT3	-	-	+++	+++	+++
	J.T30.7	EMS	OKT3	+	+	+++	+++	+++
2	J.RT-T3.1	Radiation	OKT3	-	-	+++	+++	+++
	J.RT-C305.6	Radiation	C305	-	-	+++	+++	+++
3	J.EMS-T3.3	EMS	OKT3	-	-	+++	+++	+++
	J.EMS-C305.1	EMS	C305	-	-	+++	+++	+++
4	J.RT3-T3.5	Radiation	OKT3	-	-	+++	+++	+++
	J.RT3.C305.6	Radiation	C305	-	-	+++	+++	+++

A summary of the phenotype of the indicated mutants of Jurkat as assessed by indirect immunofluorescence/flow cytometry, quantitative absorption, or antibody and complement-mediated cytotoxicity is presented (results represent at least three separate assays for each cell). Jurkat cells were mutagenized with EMS (200  $\mu$ g/ml) or gamma radiation and mutants were selected with the indicated antibody as described in Materials and Methods.

\* The relative antigen expression of the indicated mutants was compared with the expression of these antigens on the wild-type Jurkat cells, and the density of these antigens on the wild type is arbitrarily designated (+++). A designation of (+) represents an ~10-fold decrease in the level of detectable antigen assessed by flow cytometry and quantitative absorption; (-) indicates the antigen was undetectable in any of the three assays.

such clone, JT30.7 (Table II), derived by EMS mutagenization and selection with OKT3 plus complement, was studied in detail to examine the relative density of T3 and determinants detected by C305. Jurkat, J.T30.7, and J.RT-T3.1 (a T3/antigen receptor-negative cell selected for T3 negativity after radiation-induced mutagenesis) were exposed to saturating quantities of OKT3, C305, anti-Leu-5, or W6/32, followed by a fluoresceinated anti-mouse Ig, and the relative amounts of bound antibody were assessed by flow cytometric analysis (Table III). The mean channel fluorescence (MCF) obtained with J.T30.7 stained with OKT3 or C305 (57 and 63, respectively) were greater than the MCF obtained with the negative control antibody (48) but substantially less than those obtained with the wild-type, Jurkat cells (130 and 107, respectively). In other words, a mutant that expressed an intermediate density of T3 also expressed an intermediate density of determinants detected by C305. These differences in MCF obtained with J.T30.7 represent nearly a 10-fold decrease in relative fluorescence intensity obtained with Jurkat cells stained with OKT3 or C305. The MCF of the T3/receptor-negative mutant (J.RT-T3.1) stained with OKT3 or C305 did not differ from that obtained with MOPC-195, the negative control antibody (Table IV). The specificity of the changes in T3 and antigen receptor expression noted is demonstrated by the relatively equivalent amounts of T11 and HLA antigens on all three cells (Table III). These results lend further support to the notion that there is a structural requirement for the coexpression of both T3 and antigen receptor molecules and suggest that they may be stoichiometrically related on the cell surface.

TABLE III  
*Comparable Decrease in the Density of T3 and Determinants Detected by C305 on Mutants of Jurkat*

Antibody	Mean channel fluorescence*		
	Jurkat	J.T30.7	J.RT-T3.1
MOPC-195	48	48	47
OKT3	130	57	46
C305	107	63	46
Anti-Leu-5	138	129	128
W6/32	156	167	162

The indicated cells were stained with saturating amounts of the indicated monoclonal antibodies followed by a saturating quantity of fluoresceinated anti-mouse Ig. The relative fluorescence of the stained cells was determined on a FACS IV flow cytometer.

\* The MCF was determined from the fluorescence histograms obtained with the indicated cells prepared with the indicated monoclonal antibodies. Unimodal distributions were obtained with each cell preparation. A 10-fold change in relative mean fluorescence intensity would be equivalent to a change of 57 channels in MCF.

TABLE IV  
*Ability of Various Stimuli to Activate T3/Antigen Receptor-negative Mutants of Jurkat*

Stimulus	IL-2 activity (U/ml)			
	Responding cell			
	Jurkat	S.5	J.RT-T3.1	J.RT-C305.6
0	0	0	0	0
PHA	24.1	0	0	0
PMA	0	0	0	0
PHA + PMA	172	5.1	0	0
OKT3 + PMA	79.1	0	0	0
C305 + PMA	108	0	0	0
A23187 + PMA	216	98	164	150

Jurkat or the indicated mutant cells, at  $1 \times 10^6$ /ml, were cultured in the presence of the indicated stimulus for 24 h and the IL-2 activity in the resultant culture supernatants were assessed as described in Materials and Methods. Results are representative of three separate experiments.

*Failure to Detect Determinants Recognized by C305 on the Antigen Receptor Is Not Due to an Altered Epitope.* One alternative explanation for the lack of reactivity of C305 with such mutants is that the C305 antibody recognizes a conformational determinant of the antigen receptor that requires the expression of T3. As such, these determinants might not be expressed on the mutants because the antigen receptor might be present on the cell membrane in an altered conformational state. To assess this, whole cell lysates of iodinated cell surface proteins of Jurkat and such mutants were analyzed by diagonal gel electrophoresis. This approach, used by others, has demonstrated that the T cell antigen receptor is one of only a few T cell surface proteins with interchain disulfide linkages and, thus, falls below the diagonal (1). Note, whereas the individual chains of the antigen

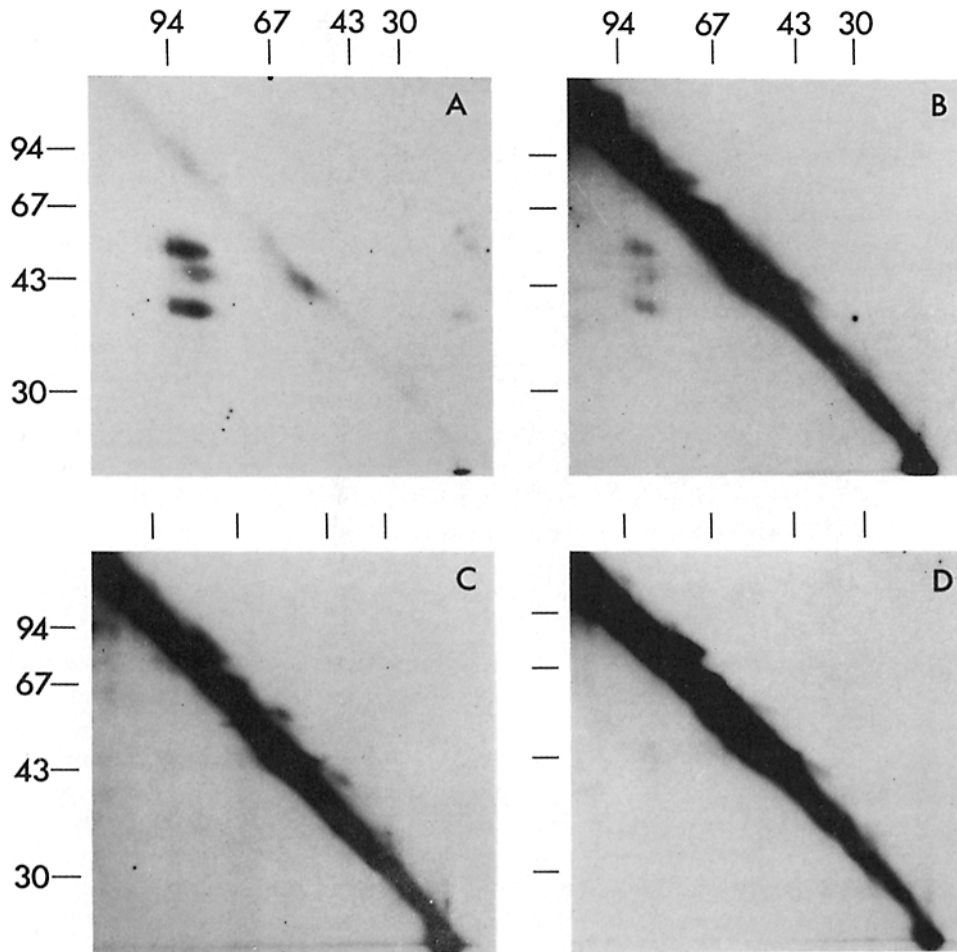


FIGURE 5. Antigen receptor molecules are undetectable among the iodinated cell surface molecules of T3/antigen receptor-negative mutants of Jurkat.  $^{125}\text{I}$ -labeled Jurkat cell surface molecules isolated with C305 (A) is compared with the iodinated cell surface proteins represented in whole cellular lysates of Jurkat (B), S.5 (C), and J.RT-C305.6 (D). SDS-PAGE was performed of samples under nonreducing conditions in the first dimension (from left to right) followed by reduction and electrophoresis in the second dimension (from top to bottom).

receptor molecule on Jurkat is demonstrated below the diagonal using the Jurkat whole cell lysate (Fig. 5B) in a position identical to the immunoprecipitate prepared with C305 and Jurkat cells (Fig. 5A), no such proteins were detectable in either S.5 (Fig. 5C) or J.RT-C305.6 (Fig. 5D). These results demonstrate that the failure to detect determinants recognized by C305 on these mutants is due to the absence of the antigen receptor on the surface of these cells and not due to an altered conformation of the antigen receptor.

*Ability of T3 and Antigen Receptor-Negative Mutants To Be Activated by a Variety of Stimuli.* Functional studies were performed to assess both the absence of T3 and the antigen receptor on these mutants of Jurkat as well as the requirement for the participation of these molecules in PHA-induced activation of Jurkat.

Representative results obtained with three of these mutants are compared with those obtained with the wild-type Jurkat cells in Table IV. All three mutants failed to produce detectable IL-2 in response to either anti-T3 plus PMA or C305 plus PMA. This is in marked contrast to the response of Jurkat to these stimuli (79.1 and 172 U/ml, respectively). Moreover, these mutants exhibited either undetectable or markedly diminished responses to either PHA alone or PHA plus PMA compared with the responses obtained with Jurkat cells (Table IV). The low level of IL-2 production by S.5 in response to PHA and PMA has been discussed in a previous report (16). The failure of these negative mutants to respond to these stimuli could not be attributed to their inability to make IL-2. In previous studies, Jurkat and S.5 cells could be activated by the calcium ionophore A23187 if PMA was also added (16). Responses nearly equivalent to those obtained with the wild-type Jurkat cells were observed when any of the mutants were stimulated with A23187 plus PMA (Table IV). These results strongly suggest that the expression of T3 and/or the antigen receptor are required for the activation of Jurkat by the lectin PHA. Moreover, the requirement for T3 and/or the antigen receptor expression can be bypassed by a calcium ionophore.

### Discussion

These studies examined the association between T3, a set of three distinct polypeptides, and the putative T cell antigen receptor, an 80–90 kD heterodimer, expressed on a human T cell line Jurkat. Anti-T3 and a monoclonal antibody reactive with idiotypic-like determinants expressed on this putative antigen receptor were used to select mutants of Jurkat that failed to express either T3 or the antigen receptor. Regardless of the mutagen used, or the antibody used in the selection, all negative mutants failed to express both T3 and such putative antigen receptor molecules. Since four separate mutagenizations were performed, a minimum of four independently derived mutants were studied. In an additional mutant, which expressed a diminished but not absent quantity of T3 determinants, the density of antigen receptor determinants was also comparably diminished, suggesting a stoichiometric relation between T3 and the antigen receptor. These results extend those of others which suggest an association between T3 and the antigen receptor (2, 9–12). They further indicate that there is an obligate requirement for the coexpression of T3 and such antigen receptor molecules on the surface of T cells.

The failure of all of the negative mutants described in this report to express both T3 and the antigen receptor suggests an intimate structural relationship between these molecules. The most direct demonstration of a close spatial association between T3 and the antigen receptor is provided by the studies of Allison and Lanier (22). In these studies, a cross-linking reagent with a 12 Å span was able to specifically cross-link T3 and the heterodimer expressed on a human T cell tumor as well as similar peptides on a murine T cell tumor.

The nature of the mutation in any of the mutants studied in this report is not clear. However, it is clear that in each mutant there is a resultant loss of expression or comparable decrease in the level of expression of both the antigen receptor and T3 antigenic determinants. Several models could account for the associated

loss of expression of both T3 and the antigen receptor: (a) Interaction between T3 and the antigen receptor might be required for these molecules to assume and maintain a stable conformation in the plasma membrane. This situation might be analogous to similar associations observed with other cell surface molecules. For example, class I major histocompatibility complex (MHC) molecules are not expressed unless  $\beta_2$ -microglobulin is synthesized (23); immunoglobulin heavy chains are not expressed on the cell membrane during B cell ontogeny until light chain synthesis occurs (24); and class II MHC antigen expression requires the expression of both  $\alpha$  and  $\beta$  chains (25). (b) A common regulatory or transport mechanism and/or some posttranslational modification may be required for the coexpression of T3 and the antigen receptor. Such a defect could account for the observed failure of expression, but it would seem unlikely that in several independently derived mutants this transport system would always be the target of the mutation. (c) A common precursor might be involved in the synthesis of both T3 and the antigen receptor. Since five polypeptide chains are involved, this model seems highly unlikely. (d) The genes encoding both T3 and the antigen receptor could be closely linked and, thus, deletion of a large section of the genetic material coding for these proteins could result in their concomitant loss. Although there are examples of this in the MHC system involving radiation-induced mutants, it seems unlikely that independently derived mutants using EMS should also result in such extensive loss of expression (26). Given the physical association that has been demonstrated between T3 and the antigen receptor in previous studies, it would seem that the first alternative of a structural requirement for insertion and stable conformation of these molecules in the plasma membrane is the most likely alternative. However, further studies using biosynthetic labeling to attempt to isolate any of these proteins that might be present in the cytoplasm of these mutants, use of the recently described cDNA probes of these genes to examine these mutants for the presence of appropriate transcripts, and fusion of mutant cells in complementation studies are required to further characterize the nature of the induced mutations. Such studies may help define the functional requirement for association between T3 and the T cell antigen receptor.

The requirement for coexpression of T3 and the antigen receptor on the plasma membrane of Jurkat may also be important in the expression of these molecules during thymocyte ontogeny. Although transcripts or even protein of the antigen receptor may be synthesized in many thymocytes, cell surface expression of such heterodimers appears to be limited to a minority of thymocytes, the more functionally mature population that expresses T3 (12, 27, 28). In keeping with these observations and the studies reported here, the expression of the T cell antigen receptor may mimic the pattern of differentiation seen in B cells; i.e., expression of membrane receptor immunoglobulin does not occur until light chain synthesis occurs (24). Thus, although the T cell antigen receptor may be synthesized, cell surface expression is delayed until the synthesis of T3 occurs.

PHA and other lectins have been used to activate T cells. In general, only immunologically competent T cells respond to such mitogens. PHA and other lectins bind to a large number of different glycosylated cell surface proteins.

However, the specific molecules involved in the activation of T cells by lectins have not been defined. The present studies demonstrate that activation of Jurkat by the lectin PHA requires the expression of T3 and/or the antigen receptor, suggesting that PHA activates Jurkat, at least in part, by binding to T3 and/or the antigen receptor. Previous studies from this laboratory (16) demonstrated that PHA induces an increase in cytoplasmic free calcium in Jurkat but fails to induce this change in the T3-negative mutant S.5. These results together with the studies reported here suggest that PHA activates Jurkat by binding to T3 and/or the antigen receptor.

Recent studies by Moretta et al. (29) have suggested that the antigen recognition capacity of T cell clones could be dissociated from T3 expression. These authors demonstrated that if T3 antigen expression was modulated off of alloreactive cytolytic T cell clones, some clones were still able to recognize alloantigens on target cells. However, from the flow cytometer profiles presented by these authors, it is clear that responsive, modulated clones have a markedly reduced density of T3 but are not truly T3 negative. In this regard, the data obtained with J.T30.7, a clone that expressed  $\sim 1/10$ th the density of T3/antigen receptor determinants that the wild-type Jurkat expressed, is of relevance. Despite the diminished density of T3/antigen receptor determinants on J.T30.7, this cell was able to be activated by anti-T3, C305, or PHA plus PMA to magnitudes comparable to those observed with Jurkat cells (data not presented). This was in marked contrast to the absent responses observed with T3/antigen receptor-negative mutants. Thus, it would appear that a diminished density of T3/antigen receptor expression may be sufficient for full cell activation and may help explain the apparent discrepancy between the observations of Moretta et al. (29) and those obtained in this report. Furthermore, studies using cells such as J.T30.7 and other mutants with even less T3/antigen receptor expression may provide a useful model for examining the minimal number of receptors required for cell activation.

It is clear that T3 and the antigen receptor are associated on the cell surface. The functional activity of this complex includes an antigen-binding capacity and transmission of one of the transmembrane signals required for T cell activation. This transmembrane signal appears to involve events associated with an increase in cytoplasmic free calcium ( $[Ca^{++}]_i$ ) (16). The relative role of T3 or the antigen receptor in mediating the effector function involved in generating this transmembrane signal is not clear. Thus, it is not clear whether the antigen receptor alone, after interaction with antigen, can induce a rise in  $[Ca^{++}]_i$  or whether an interaction with T3 is required for the signal transduction to occur. Further studies characterizing the relationship of T3 and the antigen receptor through the use of mutants, such as those described in this report, may help define the relative functions of these molecules in mediating such a transmembrane activation signal as well as defining their structural roles required for plasma membrane coexpression.

### Summary

The association between T3 and the T cell antigen receptor was examined using the T3 bearing T cell leukemic line Jurkat. A monoclonal antibody, C305,

was produced, which reacted with idiotypic-like determinants expressed on Jurkat. The molecule with which this antibody reacted was a disulfide-linked heterodimer of 90 kD, composed of polypeptides of 42 and 54 kD. Thus, C305 reacted with a molecule with characteristics of the putative T cell antigen receptor described by others. A series of mutants of Jurkat, induced with ethyl methane sulfonate or radiation, was selected for T3 or antigen receptor negativity. In every instance, there was a concomitant loss of both T3 and the antigen receptor as assessed by quantitative absorption, indirect immunofluorescence, and antibody plus complement-mediated cytotoxicity. The absence of antigen receptor molecules was confirmed on diagonal gels, excluding the possibility that conformational changes of the antigen receptor on such T3-negative mutants were responsible for the failure of such mutants to react with C305. Moreover, in a mutant that expressed a marked decrease in the level of T3 expression, there was a comparable decrease in the expression of antigen receptor determinants. These results suggest that there is an obligate requirement for the coexpression of T3 and the T cell antigen receptor. Furthermore, attempts to activate such mutants with the lectin phytohemagglutinin suggested that the expression of T3 and/or the antigen receptor was required for activation of these cells.

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