Major Histocompatibility Complex Independent T Cell Receptor-Antigen Interaction: Functional Analysis Using Fluorescein Derivatives

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Summary

We have isolated T cell receptor (TCR) cDNAs from fluorescein (FL)-specific human T cell clones ($\alpha_{FL}\beta_{FL}$), and transferred them to TCR β^- Jurkat cells in order to study direct FL-binding to the TCR. Using either FL-conjugated polymers (FL-polymer) or FL-substituted Sepharose beads, we are able to demonstrate the direct binding of antigen to the T cell surface, and the functional activation of the T cell transfectants. We present evidence against the involvement of major histocompatibility complex (MHC) molecules or antigen presentation in the interaction of FL with the $\alpha_{FL}\beta_{FL}$ transfectants. Additionally, we have examined the effect of ring substitutions on the FL molecule as well as specific alterations of substituents attached to the 5' position, and we have found that all of them interfere with the functional recognition of the $\alpha_{FL}\beta_{FL}$ TCR. These experiments demonstrate that TCRs like antibodies have intrinsic affinities for antigen, even without the involvement of MHC molecules.

Molecular (reviewed in references 1 and 2) and cellular (3–7) studies have identified the TCR for antigen as a 90 kD α/β heterodimer on the T cell surface. The primary function of TCRs is in the recognition of antigen in a complex process that involves gene products of the MHC on APC (8, 9). An important function of these MHC molecules is to bind and present peptide fragments of protein antigens for recognition by TCR. Defined peptides which represent appropriate epitopes in foreign antigens will selectively bind particular MHC molecules (10, 11). These peptides, when presented by APC, will activate T cell clones (12) or transfectants expressing TCRs of those clones in an antigen specific and MHC-restricted fashion (13, 14). MHC molecules also interact with CD4 (class II MHC) or CD8 (class I MHC) cell-surface receptors on T cells in a manner which promotes adhesion of the interacting cells and signal transduction (15–18). Transfection of antigen specific and MHC-restricted TCRs into host cells of irrelevant specificity results in the transfer of specificity for both MHC and antigen (19–21). These results demonstrate that the TCR is the only molecule necessary to establish antigen specificity and to confer MHC restriction on a T cell.

There are few reports which demonstrate that antigen can interact with TCR in a manner that is independent of MHC (22–25). A novel series of murine (26) and human (27) T cells which recognize the hapten fluorescein (FL)[1] were generated to determine if the TCR has the intrinsic capacity to bind antigen independent of MHC and accessory cells. These clones were shown to interact with polymers substituted with multiple FL molecules independently of accessory cells. Several of the human FL-specific T cell clones can be activated to secrete IL-2 by incubation with Sepharose beads conjugated with FL-isothiocyanate (FITC; reference 27). Significantly, solubilized forms of the surface TCR from these clones specifically bound to FITC-substituted Sepharose beads in affinity chromatography experiments (27). However, whether antigen recognition and functional cellular adhesion of these clones could occur in an absolutely MHC independent fashion was unclear. To address this issue, a transfection model using a functional T cell tumor with the TCRs from the FL-specific T cell clones was developed to study the direct binding of antigen to the TCR in the absence of APC

[1] *Abbreviation used in this paper:* FL, fluorescein.

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and accessory molecules. We present the results of one such cellular transfection system which demonstrate that in this system the TCR can engage the hapten FL under defined circumstances without MHC interaction. In addition, we have been able to define several parameters of the TCR's ability to recognize the FL molecule by examining the effect of chemical substitution on the FL ring system in a direct binding and activation assay with transfected cells. This system may be useful in the eventual determination of the precise antigen binding site of a TCR.

Materials and Methods

Cell Lines and FL-Polymers. The cell lines shown in Table 2 were propagated in RPMI-1640 (JRH Biosciences, Lenexa, KS) with the standard additions which include glutamine, Pen-Strep, and 10% FCS (JRH Biosciences). Transfectants were routinely grown in RPMI-1640 (JR Scientific) medium containing Hygromycin B (Sigma Chemical Co., St. Louis, MO) at 0.5 mg/mL and/or G-418 (Gibco Laboratories, Grand Island, NY) at 0.5 mg/mL. CTLL-2 cells (American Type Culture Collection, Rockville, MD) were grown in RPMI-1640 as described above with the added supplementation of 50 μM 2-ME and 10 μ/mL rIL-2 (Cetus Corp., Emeryville, CA).

The properties of the FL-Polymers used in this publication are summarized in Table 3 below. The preparation of FL-conjugated polyacrylamide, dextran, or Ficoll polymers has been previously described in detail (26, 28, 29).

cDNA Cloning. T cell clones RFL 3.8 and 3.51 were stimulated by FITC as previously described (27), and grown to a density of 10^6 cells/mL. Poly A^+ RNA was obtained by standard methods after 2x binding to Oligo dT Cellulose (T7, Collaborative Research, Waltham, MA). 5 μg of the poly A^+ RNA was used in the first strand synthesis reaction, which was immediately followed by the second strand synthesis protocol of Gubler and Hoffman (30). The double stranded cDNA was made blunt ended by T4 DNA Polymerase, Eco RI linkers were attached after methylation by Eco RI methylase, and the cDNA was size fractionated according to standard methods (31). 0.2 μg of cDNA was ligated to 1 μg Eco RI cleaved and purified λ gt-10 arms (72) overnight at 12°C. Half of the ligated DNA was packaged into λ phage packaging extract (Stratagene™, La Jolla, CA) for 2 h at room temperature, and it was inactivated by the addition of 0.5 mL TM (10 mM Tris-HCl, pH 7.5 and 10 mM $MgCl_2$) and 10 μL chloroform. The phage was titrated on both permissive (HFL) and restrictive (C600) strains of bacteria, and a library of 1.25×10^6 pfu was obtained. The cDNA library from RFL 3.8 and 3.51 were plated on square dishes (24.5 cm × 24.5 cm × 2 cm) at a density of 10^5 phage/plate. The nitrocellulose lifts were screened using either the human TCR Cβ gene segment (32) or the Cα (33) gene segment. We found 1 positive plaque per 5,000 pfu for both α and β cDNAs. The purified Eco RI inserts from the λ gt-10 phage minipreparations were subcloned into pCU12. The cDNA's for both the α and β chain genes were sequenced using a combination of gene-specific and MP18 sense and antisense primers. The amino acid sequence of all four chains is presented in Fig. 1 *F*, together with the 30% conserved sequences accumulated from 26 TCR cDNA's (34).

Northern and Southern Analysis. The variable segments of each of the α and β cloned TCR cDNAs were subcloned, and used as probes for both Northern and Southern hybridization. cDNA fragments from the DP, DQ, and DR β genes were cut out from pBR322, and purified over 1.2% agarose gel (obtained from L. Gadulis, and B. Wallace, HLA Laboratory of the City of Hope Medical Center). 10 μg of cellular DNA was digested with restriction enzymes as indicated, and electrophoresed on 1% agarose gels in TBE (pH 8.3) buffer (35). RNA samples (30 μg) were electrophoresed on 1–1.5% agarose gels containing formaldehyde using MOPS (pH 7.5) as a running buffer (35). Transfers were accomplished by capillary action of 20× SSC (pH 7.5) buffer onto nitrocellulose sheets (BA85; Schleicher & Schuell, Keene, NH). The nitrocellulose sheets were visualized by long wavelength UV light to assess the evenness of the transfer of ethidium bromide stained nucleic acids. The sheets were then further processed according to standard methods (35), and after 12 h of pre-hybridization, 5×10^5 cpm of denatured probe (^{32}P-labeled) was added to the bag in hybridization buffer containing 10% dextran-sulphate. After 12–14 h of hybridization, the blots were washed using SSC as low as 0.5×, and put up to film for times ranging from 12 h to 5 d using Cronex Lightning Plus Screens (Dupont).

cDNA Transfection in Eukaryotic Expression Vectors. To express TCR cDNAs, we inserted them into expression vectors which included the EBV virus OriP gene segment (36). The vectors shown in Fig. 1 *G* replicate as episomes in primate cells (37). The ability to replicate depends on the interaction of the EBNA 1 protein with OriP gene segment on the episome (38). The CMV I-E promoter (kindly given by Drs. Pande and Zaia, City of Hope Medical Center) was cloned into the polylinker of both the p266.1 and p220.2 plasmids (kindly given by Dr. W. Sugden, McCardle Laboratory) to obtain high expression in human cell lines. The α and β chain TCRs were cloned into p266.1 and p220.2 respectively, and the junctional sequences determined (data not shown). The correct orientation of the TCR cDNA containing vector was used in transfection.

Electroporation was carried out using a modification of a procedure developed for human PBL and T cells (39). Briefly, cells were split the night before transfection to a density of 0.3–0.4 × 10^6/mL. The following day cells were concentrated to 2×10^7 cells/mL in RPMI-1640 containing 10% FCS (complete medium), and 0.4 mL were suspended in an electroporation cuvette together with plasmid DNA at 100 μg/mL. The suspended cells were incubated on ice for 10′, and then 1 pulse of electroporation was carried out at 200–250 V and 800–960 Farad (BRL Cell-Porator). The shocked cells were left on ice for an additional 10′, then pipetted and washed in complete medium. After 2 d of recovery, the living cells were separated by centrifugation through Ficoll and plated in 96-well plates in Hygromycin B (0.5 mg/mL) or Hygromycin B + G-418 (0.4 mg/mL + 0.5 mg/mL) to select for transfectants. Typically, we found a frequency of 1 in 10^{+4} transfectants using this procedure. Positive clones were identified initially by growth in selection medium, and then expanded for FACS and Northern analysis.

FACS Analysis. We examined 50 clones containing either the RFL3.8 or RFL.3.51 β chain by FACS using a mAB against CD3 (RW2-8C8; reference 40) and staining with an FITC conjugated goat anti-mouse secondary antibody (Cappel Laboratories, Durham, NC). The cell staining procedure was according to Siliciano et al. (27), and propidium iodide (PI, 1 μg/mL) was added to enable exclusion of dead cells. Cells were analyzed on a Becton-Dickinson FACS IV equipped with dual lasers (argon and UV). Both 90° light scatter and the fluorescence channel (510 nM) were used to gate the single cell population that was free of PI. The representative plots shown in Fig. 2 were obtained from analysis of 2×10^4 cells. Fluorescent microspheres were used to correct changes in amplitude in the fluorescence channel when comparing samples analyzed on different days. In general, the expression of the RFL3.8

β chain clones is 4–5× greater than from the RFL3.51 β chain in combination with the endogenous Jurkat α chain (unpublished data). However, Northern analysis of a series of RNA samples from the FACS analyzed clones did not reveal any significant difference in the RNA expression (unpublished data). Others have found a difference in TCR pairing ability between chains when the RNA levels were similar (41).

In the case of the FL-Polymers, 10^6 cells to be analyzed were washed twice in WM [HBSS containing 2% newborn calf serum (JRH Biosciences), 10 mM Hepes (pH 7.4), 0.1% glucose, and Penn-Strep]. The various FL-Polymers were added to the cells in 50 μL of the same buffer at a final concentration of 30 μg/mL. The cells were incubated in an ice bath for 30', then washed twice in WM, and finally resuspended in 0.5 mL of WM containing propidium iodide at 1 μg/mL. The samples were kept ice cold until analysis, typically in <2 h, otherwise the background staining would rise dramatically. As with the antibody analysis, 2×10^4 cells were analyzed per profile.

Cell sorting was accomplished with the aid of a Cicero analysis system (Cytomation) on the FACS IV, and using standard techniques of cell preparation using azide-free medium. The brightest 5% of cells were collected, expanded, and resorted to obtain a relatively homogeneous population (see Fig. 2).

Cell Activation Assays. 0.6×10^6 cells to be treated with agents (FL-Polymer, etc.) were washed twice to remove antibiotics, and resuspended in one well of a 24 well plate (Falcon) with 0.6 mL normal growth medium. The cells were incubated with the agents for 24 h, and the supernatants collected by centrifugation and stored at 4°C. FL-Polymers were added at 1 μg/mL with or without 1 or 10 ng/mL PMA. Sepharose beads in a volume of 20 μL (determined to be saturating by titration) were added to the cells to be stimulated for 24 h. FL-Cad was added as dilutions of a dimethylformamide stock solution of 100 mM.

CTLL-2 cells are cultured in 4U/mL recombinant IL-2 (Cetus Corp.), and every 3-4 days they are restimulated with fresh IL-2. The cells to be tested are washed four times, and resuspended in growth medium containing 50 μM mercaptoethanol. 10^4 CTLL were cultured in 100 μL medium in 96-well plates (NUNC) with dilutions of IL-2 containing supernatants up to 50%, or a series of rIL-2 dilutions, 0.5 or 5 ng/mL PMA, or medium without additions. Each supernatant was tested in triplicate, and each experiment was repeated twice or more. The data presented is an average of all the experimental determinations for each point, with no separate determination having a standard error of >20% from the mean.

Preparation of Sepharose Beads Substituted with FL Derivatives. We followed the techniques described in (27), except for the following modifications. 0.5 gram of CNBr Activated Sepharose 4B (Pharmacia) was washed according to the manufacturer, and reacted with a 100× excess of 1,6 diaminohexane in 0.1 M $NaHCO_3$ (pH 8.3) for 2 hours, then washed again 2× with coupling buffer. Either a 1 or 8 mM solution of FITC or X-isothiocyanate was reacted with the beads in 0.1 M borate buffer for 60' at 37°C. Alternatively, when the FL derivative was in the amine form, it was directly reacted with the Sepharose beads without the previous spacer coupling. The ligand was washed away, and the beads were neutralized according to the manufacturer. Ethanolamine was reacted directly with the Sepharose beads, and they were neutralized. The beads were stored at 4°C under foil in PBS. Each batch of derivatized bead was adjusted in concentration to 2×10^6 beads/mL. All of the beads are greater in size than 50 μM. The FL derivatives containing primary amines were all obtained from Molecular Probes (Eugene, Oregon), while some of the isothiocyanate derivatives were also obtained from Sigma Chemical Co.

Results

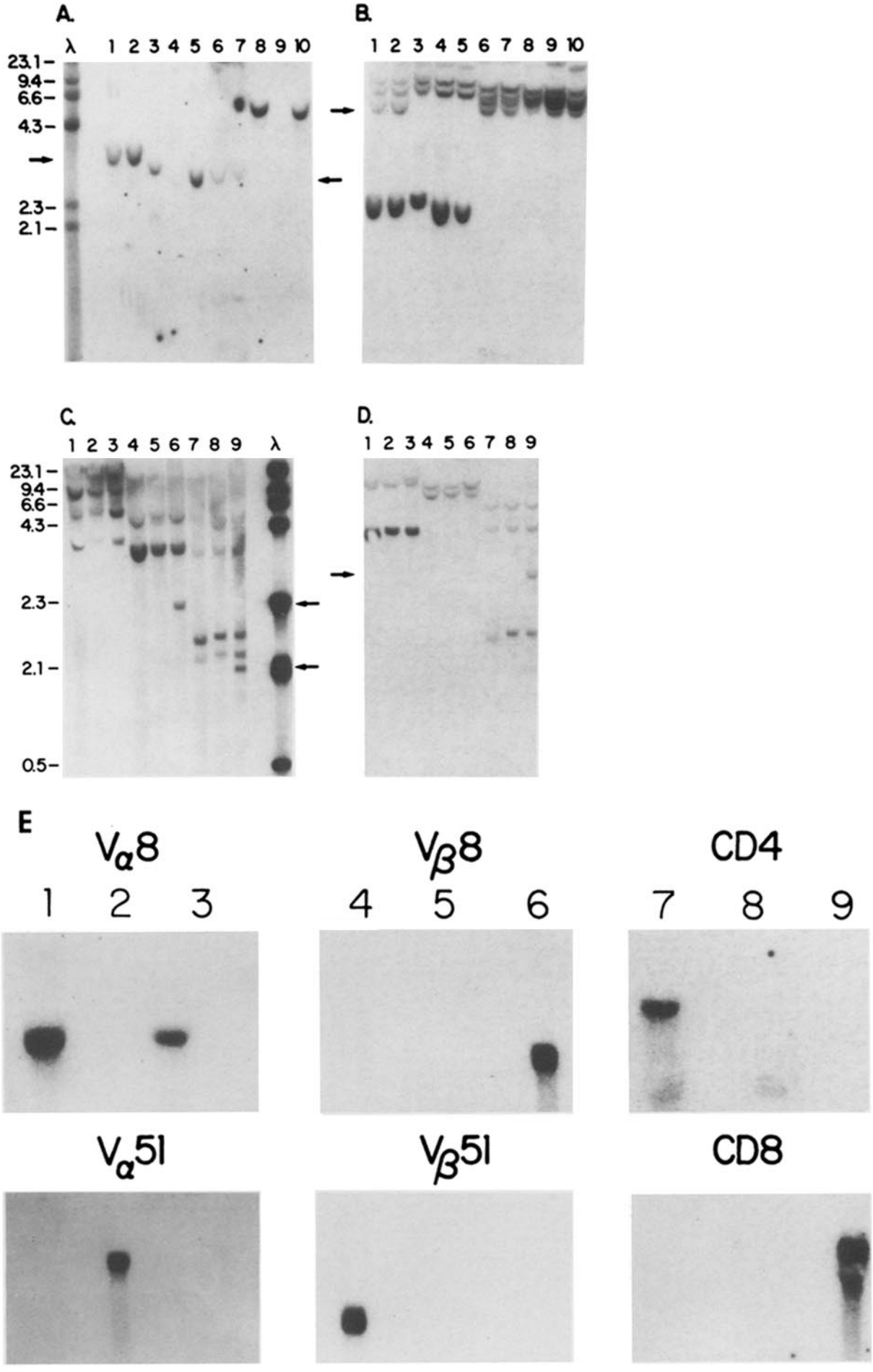
Cloning and Characterization of the $\alpha_{FL}\beta_{FL}$ TCRs. As a first step in understanding the structural interactions between FL and the TCR, we cloned the TCR α and β chains from cDNA libraries constructed from two human T cell clones referred to as RFL 3.8 ($CD4^+$) and RFL 3.51 ($CD8^+$, Fig. 1 *A–F* and reference 27). We made V-J segment probes from each of the 4 cDNAs, and hybridized them to various genomic DNAs (Fig. 1 *A–D*). Each of the 4 V-J segment probes hybridized to a cell-specific rearranged band in the appropriate DNA sample. For instance, the RFL 3.8 α cDNA V-J segment hybridized to a 3.5 kb Eco RI and a 3.2 kb Hind III fragment in DNA from clone RFL 3.8 and a related $CD4^+$ FL-specific T cell clone RFL 3.1 while DNAs from other human T cell and non-T cell lines had hybridizable bands at different positions (Fig. 1 *A*, compare lanes *1* and *2* versus 3–5 and lanes *6* and *7* versus lanes *8–10*). Note, that Jurkat DNA has deleted the V segment that encodes the FL-specific V_α gene segment (Fig. 1 *A*, lanes *4* and *9*). In each case, when an FL-specific V-J probe was hybridized to various test DNAs, only the expected cell line from which the V-J segment was originally cloned demonstrated rearrangement.

The human gene segments used in each of the 4 cDNAs are shown in Table 1. The α chain from RFL3.8 contains a V gene segment which is almost identical to $V_\alpha 17$ (2), while the J segment was identical to a previously identified J segment termed F in the recent nomenclature of (2). Additionally, the β chain from RFL 3.8 uses a V gene segment that is similar to other members of the V_β 13.2 family, although there are significant (16/92) amino acid differences (2). In the case of clone RFL 3.51, the α chain contains a V segment that is similar to the $V_\alpha 2$ family, while the J segment (L) has been previously identified (2). The β chain from RFL 3.51 uses a new member of the $V_\beta 2$ gene family that we have designated 2.5. The sequence of each of the individual chains is shown in Figure 1F. There is a striking similarity

Table 1. *Gene Segments Used by FL-specific T Cell Receptors*

Clone	Phenotype	Specificity	Chain	V	D	J	C
RFL 3.8	$CD4^+CD8^-$	FL-Class II	α	17	-	F	C_α
			β	13.2	1	1.5	$C_\beta 1$
RFL 3.51	$CD4^-CD8^+$	FL-Class I	α	2.8	-	L	C_α
			β	2.5	1	1.5	$C_\beta 1$

Summary of the gene segments used in the formation of the TCR cDNAs cloned from the FL-specific T cell clones shown on the left side of the table (27). The symbols V,D,J, and C are defined in the legend to Fig. 1. CD4 and CD8 expression were determined from both antibody (27) and Northern analysis (Fig. 1 *E*).


A.
λ 1 2 3 4 5 6 7 8 9 10
23.1–
9.4–
6.6–
4.3–
2.3–
2.1–
B.
1 2 3 4 5 6 7 8 9 10
C.
1 2 3 4 5 6 7 8 9 λ
23.1–
9.4–
6.6–
4.3–
2.3–
2.1–
0.5–
D.
1 2 3 4 5 6 7 8 9
E
Vα8
Vβ8
CD4
1 2 3
4 5 6
7 8 9
Vα51
Vβ51
CD8

F

TCR α_{FL} CHAIN VARIABLE REGION SEQUENCES

```
      -10         0         10        20        30
       |          |         |         |         |
.............................QQVTQ.P..LSVQPGA.VTL.   30% CONSERVED
MDKILGASFLVLWLQLCWVSGQQKEKSDQQQVKQSPQSLIVQKGGISIIN   RFL 3.8
MKWLRVLLVI-LWLQLSWVWSQQKE------VEQNSGPLSVPEGAIASLN   RFL 3.51
————————L————————————————————V————————

       40         50        60        70        80
       |          |         |         |         |
CNYSTS.S.YLFWYRQ.PG.GPQLLI.I.SN.......R...LF.K..K   30% CONSERVED
CAYENTAFDYFPWYQQFPGKGPALLIAIRPDVSEKKEGRFTISFNKSAK   RFL 3.8
CTYSDRVSQSFFWYRQYSGKSPELIMSIYSN-GSKEDGRFTAQLNKASQ   RFL 3.51
————————————————V————————————————

       90        100       110       120
       |          |         |         |
SFSL.ITASQP.DSAVYFCA.....SG.KLIFG.GTRLSV.P           30% CONSERVED
QFSLHIMDSQPGDSATYFCA-ASFSGNTPLVFGKGTRLSVIA           RFL 3.8
YVSLLIRDSQPSDSATYLCAVNGVSDGQKLLFARGTMLKVEP           RFL 3.51
————————V————————————J————————
```

TCR β_{FL} CHAIN VARIABLE REGION SEQUENCES

```
      -10         0         10        20        30
       |          |         |         |         |
...................A.VTQTPR..V...GQ.VTLRC.PISGH..   30% CONSERVED
MSLGLLCCGVFSLLWAGPVNAGVTQTPKFRVLKTGQSMTLLCAQDMNHE-   RFL 3.8
MLLLLLLLLLLLGPGSGL-GAVVSQHPSRVICKSGTSVKIECRSLDFQAT   RFL 3.51
————————L————————————————V————————

       40         50        60        70        80
       |          |         |         |         |
..FWYRQ.PGQGLELL.YFN.......DDSG.P.DRFSASRPEGS.STLK   30% CONSERVED
YMYWYRQDPGMGLRLIHYSVGEGTTAKG-QV-P-DGYNVSRLKKQNFLLG   RFL 3.8
TMFWYRQFPKKSLMLMATSN-EGSKATYEQGVEKDKFLINHASLTLSTLT   RFL 3.51
————————————————V————————————————

       90        100       110
       |          |         |
IQSAEPGDSAVYLCASSL.G.TQYFGPGTRLTVL                   30% CONSERVED
LESAAPSQTSVYFCASRTATQPQHFGDGTRLSIL                   RFL 3.8
VTSAHPEDSSFYICSARVPGEPQHFGDGTRLSIL                   RFL 3.51
————————V————————D-J————————
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G

EBV BASED EXPRESSION PLASMIDS
WHICH ARE MAINTAINED AS EPISOMES IN HUMAN CELLS

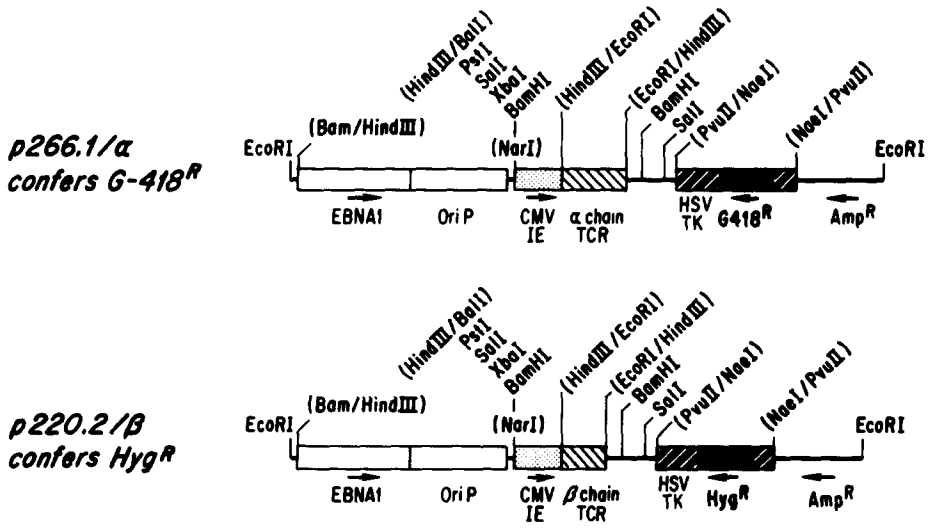


Figure 1. Cloning and analysis of α_{FL} β_{FL} TCRs. (*A*) Rearrangement of the variable segment gene used in the assembly of FL-specific TCRs: Detection by variable gene-specific probes. The $V_\alpha 17$ gene segment from the T cell clone RFL3.8 was labeled with random hexamers and α-^{32}P [dCTP] using the Klenow enzyme. Genomic DNA samples from T cell clone RFL3.1 (lanes *1* and *6*), RFL3.8 (lanes *2* and *7*), autologous EBV transformed cells from (lanes *3* and *8*), Jurkat T cells (lanes *4* and *9*), and HeLa cells (lanes *5* and *10*) were cut with either Eco RI (lanes *1–5*) or Hind III (lanes *6–10*) and probed with the $V_\alpha 17$ gene segment. (*B*) The blot in (*A*) was reprobed with the $V_\beta 13.2$ gene segment from the T cell clone RFL3.8. (*C*) The $V_\alpha 2.8$ gene segment from the T cell RFL3.51 was labelled as described in (*A*). DNA from Jurkat (lanes *1*, *4*, and *8*), RFL3.8 (lanes *2*, *5*, and *8*), and RFL3.51 (lanes *3*, *6*, and *9*) was cut with Bam HI (lanes *1–3*), Hind III (*4–6*), or Xba I (lanes *7–9*) and probed with the V_α 2.8 gene segment. (*D*) The blot shown in (*C*) was reprobed with the $V_\beta 2.5$ gene segment from T cell clone RFL3.51. In each of the panels the arrow(s) point to the location of the rearranged band. (*E*) Northern analysis of the RNA prepared from FL-specific T cell clones and the Jurkat cell line. RNA prepared from T cells such as RFL3.8 (lane *1*, *6*, and 7), RFL3.51 (lane *2*, *4*, and *9*), RFL3.4 (lane *3*), and Jurkat (lane *5* and *8*) was hybridized with the indicated probes from the T cell clones RFL 3.8 ($V_\alpha 8$ or $V_\beta 8$) and RFL 3.51 ($V_\alpha 51$ or $V_\beta 51$) or from the CD8 or CD4 genes (kindly provided by D. Littman, University of California at San Francisco). (*F*) Sequences were obtained from cDNAs subcloned into PUC 12. The protein domains are signified as the letters L (leader peptide), V (Variable region), D (Diversity Region), and J (Joining Region). The dashes give the best alignment based upon the position of cysteine residues. The 30% conserved sequence is from Kabat et al. (34). The placement of periods indicates <30% sequence conservation. (*G*) Vectors used to express the $\alpha_{FL}\beta_{FL}$ TCR cDNAs in T cells. The Cytomegalovirus Immediate-Early Promoter-Enhancer (gift of Drs. Pande and Zaia, City of Hope Medical Center) was cloned as a Hind III fragment, followed by the cloning of the TCR α or β cDNAs.

in that both TCRβ cDNAs use the same Jβ, and a region in both TCR α cDNAs between aa 67–74 shows similarity where most TCRs are divergent. Finally, Northern analysis further confirms the specificity of the V-J segments for the individual T cell clones, as well as the absence of minor species of contaminating TCR RNAs (Figure 1 *E*).

Transfection and FACS Analysis. We subcloned both pairs of FL-specific TCR cDNAs into a novel series of expression vectors based upon the Epstein-Barr virus (EBV) (Figure 1 G). The expression of the EBNA I gene allows the OriP gene segment to function as an origin of replication, and as a result the plasmids are maintained as episomes in human cells (36). The vectors were introduced into the TCR β^- mutant of Jurkat, 31-13 (42) by electroporation (39). Single colonies were selected and expanded in Hygromycin containing medium, and analyzed by fluorescence activated cell sorting (FACS) to determine whether the introduction of a β TCR cDNA had restored expression of surface CD3 expression (42, 43). Figure 2 shows that compared to the parental line Jurkat, 31-13 is negative for CD3. In contrast, the single transfectants 3C11 or 42 (Table 2) which express β_{FL} TCR chains from clones RFL 3.8 and RFL 3.51 respectively, have almost completely restored surface CD3 expression. Subsequently, we introduced the vector p266.1/α (Figure 1G) carrying the α_{FL} TCR chain of clone RFL 3.8 into the cell line 3C11 and administered the dual selection of G-418 and hygromycin. Surprisingly, we observed colonies within 1 week, at a frequency 100 fold higher than in the initial transfection with p220.1/β (Figure 1 G). This may be the result of the already established EBV plasmid supplying EBNA I in trans to the incoming plasmid which has its own OriP site (37).

In order to determine if both the α_{FL} and β_{FL} TCR chains were being expressed, individual drug-resistant clones were expanded, and subsamples were stained with the FITC containing polyacrylamide polymer, $FL_{790}PA_{2300}$ (see Table 3 for a description of the FL-polymers and Table 2 for the cell transfectants) and analyzed by FACS (Fig. 2). Only cell lines such as A5A6 (Table 2) which contain both the α and β TCR chains from clone RFL 3.8 bound polymer, while the parental line and other transfectants which contained just a β_{FL} chain were negative. The degree of binding of the FL-Polymer to the $\alpha_{FL}\beta_{FL}$ cell lines is reduced, because there are two types of TCR on the cell surface. One type is the functional

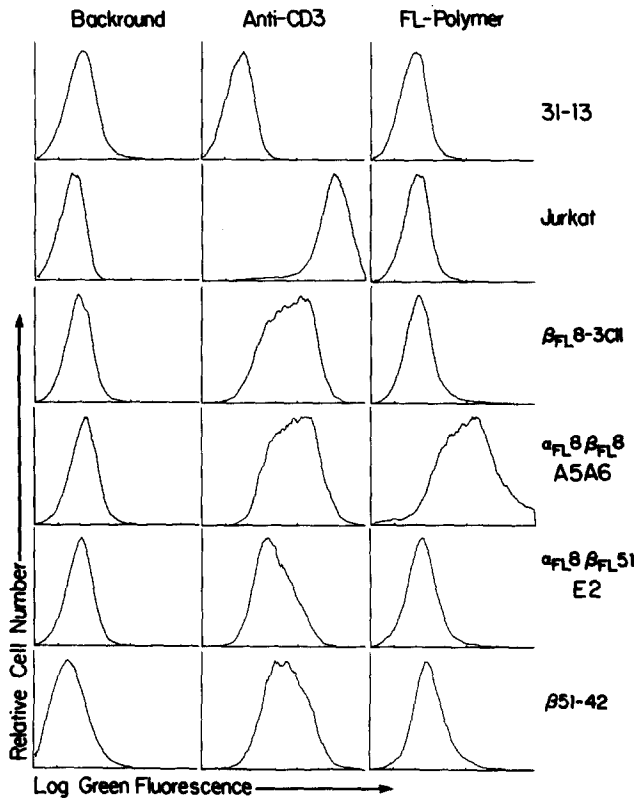


Figure 2. FACS profiles of T cell lines using anti-CD3 or the FL-polymer. (*A*) The cell lines listed on the right-hand side of the figure were first treated (2×10^4) with either medium (background), OKT3 (CD3), or $FL_{790}PA_{2300}$ (FL-Polymer) as described in Materials and Methods. The cells were then reacted with fluoresceinated and affinity-purified goat anti-mouse 2° antibody (Background and CD3). There was no secondary reaction with the cells stained with the FL-Polymer. A description of the cell lines can be found in Table 2 and in the text.

Table 2. *Cell Line Properties*

Cell line	TCR chains	Selection conditions	Reference
Jurkat (J77)	α_J and β_J	None	Alcover et al. 1988
31-13	α_J	None	Alcover et al. 1988
3C11N	α_J and $\beta_{FL}8$	Hygromycin	This paper
43N	α_J and $\beta_{FL}8$	Hygromycin	This paper
A5A6	α_J and $\alpha_{FL}8$ + $\beta_{FL}8$	Hygromycin + G-418	This paper
1C5	α_J and $\alpha_{FL}8$ + $\beta_{FL}8$	Hygromycin + G-418	This paper
E3E4	α_J and $\alpha_{FL}8$ + $\beta_{FL}8$	Hygromycin + G-418	This paper
42	α_J and $\beta_{FL}51$	Hygromycin	This paper
E2	α_J and $\alpha_{FL}8$ + $\beta_{FL}51$	Hygromycin + G-418	This paper

Table 2 summarizes the properties of the cell lines used in this publication. Details of the derivation of the transfectant cell lines and their growth conditions can be found in Materials and Methods.

Table 3. *Properties of FL-Polymers*

Designation	Polymer backbone	Average mol wt ($\times 10^{-5}$)	Antigen valence (FL groups/molecule)	Epitope density (mol FL/g polymer $\times 10^4$)
$FL_{790}PA_{2300}$	Polyacrylamide	23.0	790	2.91
$FL_{1000}PA_{2500}$	Polyacrylamide	25.0	1,000	2.50
$FL_{95}PA_{300}$	Polyacrylamide	3.0	95	3.16
$FL_{1000}Dex_{2500}$	Dextran	25.0	1,000	2.50
$FL_{110}Dex_{330}$	Dextran	3.3	110	3.00
$FL_{65}Fic_{220}$	Ficoll	2.2	65	3.38

Techniques of analysis to determine average molecular weights of polymer preparations were done as previously described (26). FL content of polymers was measured as described in (27).

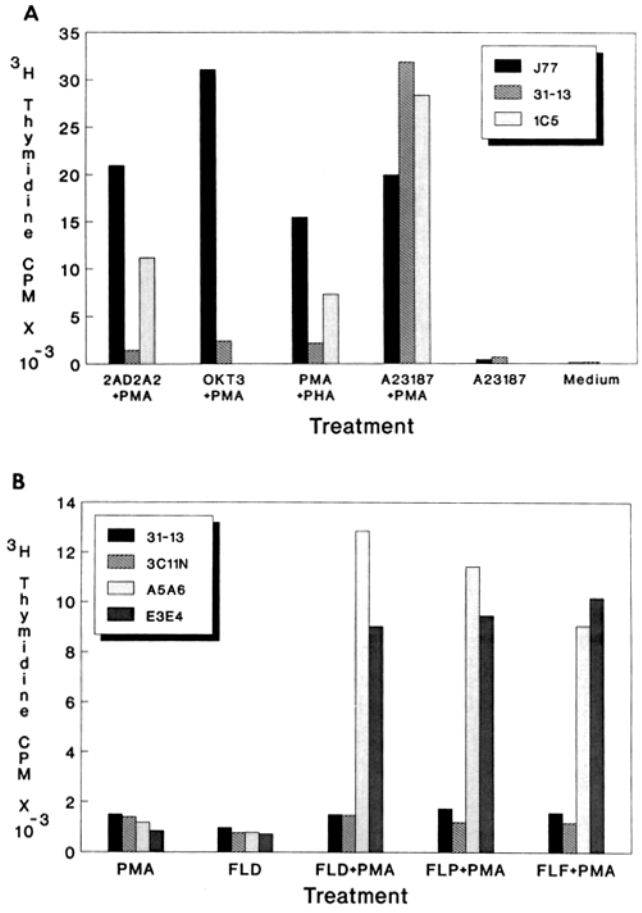


Figure 3. Proliferation assays using supernatants from T cell lines activated by FL-polymer reagents. (*A*) Antibody and Calcium Ionophore stimulate IL-2 production in T cells. The T cells indicated in the box were treated with the reagents indicated on the bottom of (*A*) for 24 h as described in Materials and Methods. 50% supernatants were incubated with CTLL-2 cells for a total of 24 h including a 6-h pulse with ^{3}H-thymidine (1 μCi). The cells were harvested with the aid of a semi-automatic cell harvester apparatus (Skatron Inc., Sterling, VA). The bars represent data collected from at least two experiments with each treatment done in triplicate (n = 6) with an SE of <20%. The cell lines are described in Table 2. (*B*) FL-polymer treatment of T cell transfectants. The cell lines indicated in the box on the left of (*B*) were treated with the indicated reagents (*bottom*) as described in (*A*). The FL-polymers FLD ($FL_{1000}Dex_{2500}$), FLP ($FL_{1000}PA_{2500}$), or FLF ($FL_{65}Fic_{220}$) are described in Table 3.

$\alpha_{FL}\beta_{FL}$ TCR, while the other is the mixed TCR containing α_{FL} + β_J which is non-functional as is the case in the cell line 3C11N (Fig. 2). Our analysis of the TCRs from clone RFL 3.51 has been hampered, because the β chain is unstably expressed in transfected 31-13 cells, so we are unable to test whether the $\alpha\beta$ heterodimer from RFL 3.51 binds FL. It is clear in the cell lines we tested that the α or β TCR cDNA from RFL 3.8 alone or in combination with other TCR chains does not confer FL binding (Fig. 2, compare clones 3C11 or E2 with A5A6). Therefore, for clone RFL 3.8, we assume that both the α and β TCR chains contribute to antigen binding.

Stimulation of IL-2 Secretion by FL. As reported previously, FL-Polymer binding to the T cell clone RFL 3.8 caused the production of IL-2 (27). The transfection system presented here allows us to determine unequivocally whether there is a necessity for cellular presentation of the FL-Polymer in order to stimulate the transfectant to produce IL-2. Since the TCR β_J (Table 2) reconstituted 31-13 cells make IL-2 (41, 43), we first determined whether the $\alpha_{FL}\beta_{FL}$ cell line would also make IL-2 (Fig. 3 *A*) when challenged with a mAB against CD3 or with a combination of the calcium ionophore A23187 and PMA (44–46). We used a different $\alpha_{FL}\beta_{FL}$ cell line called 1C5 in this experiment (see Table 2 for a description of the transfectant cell lines), and found that 1C5 and Jurkat cells responded similarly to these stimuli. Subsequently, we incubated the cells shown in Fig. 3 *B* with FL-Polymers with and without PMA for 24 h, and quantitated the IL-2 production by CTLL assay. Clearly, the FL-Polymer in combination with PMA specifically activates the $\alpha_{FL}\beta_{FL}$ cell lines A5A6 and E3E4 (Table 2), although the FL-Polymer without PMA was not able to activate the $\alpha_{FL}\beta_{FL}$ T cells to make IL-2 (Fig. 3 *B*). Cell lines 3C11N and 31-13 did not respond to FL-Polymer, regardless of the polymer backbone (see Table 3). However 31-13 (Fig. 3 *A*) or 3C11N (data not shown) are capable of being activated to make IL-2 when incubated with calcium ionophore + PMA which bypasses the requirement for triggering through the surface TCR (42). These results explicitly show that only the $\alpha_{FL}\beta_{FL}$ transfectants will respond to FL-Polymers, whereas other T cells, although capable of making IL-2 (Figure 3 *A*) are incapable of functionally recognizing the FL antigen.

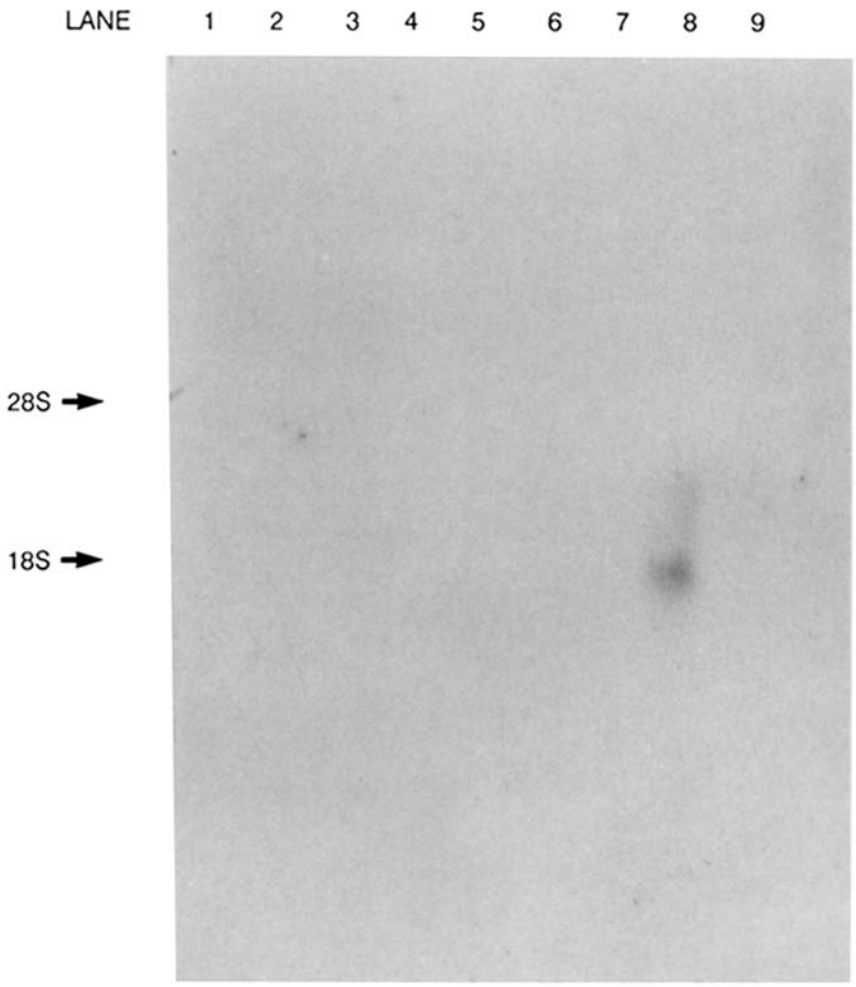


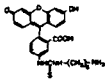
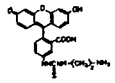
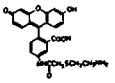
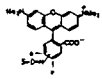
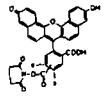
Figure 4. Northern analysis of RNA from transfected T cells. 30 μg of RNA was denatured and electrophoresed on a 1.5% agarose gel containing MOPS buffer as described in Materials and Methods. The RNA which was transferred to nitrocellulose was probed with a mixture of all three DP, DQ, and DR β Class II MHC cDNA probes in equal proportion. The blot was washed using the same conditions as in Fig. 1 *E*, and exposed to film for 48 h. RNAs are from cell line A5A6 (lane *1*), E2 (lane *2*), Jurkat (lane *3*), 1C5 (lane *4*), 3C11N (lane *5*), 31-13 (lane *6*), Jurkat (lane 7), RSEBV (lane *8*), and 31-13 (lane *9*). RNA made from cell lines induced for 24 h with 1 μg/mL $FL_{1000}DEX_{2500}$ and PMA is shown in lanes *1–6*, while uninduced cellular RNA is shown in lanes *6–9*.

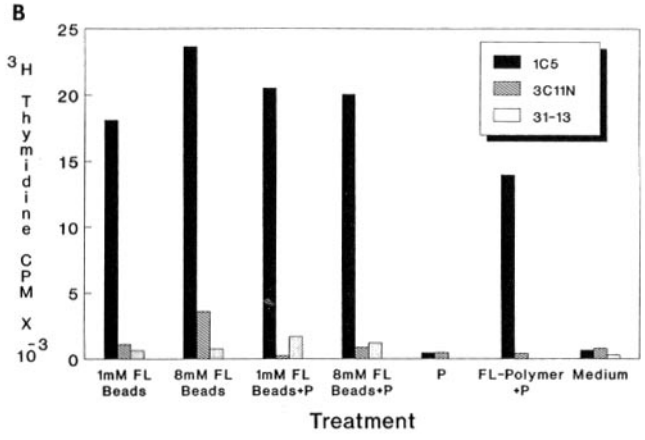
Inability to Detect MHC Class II Gene Expression in Transfected Cells by Northern Analysis. The direct binding studies described in the preceeding section showed that FL-Polymers which bind to the $\alpha_{FL}\beta_{FL}$ transfectants (Figure 2) could also functionally activate the T cells to produce IL-2. To determine whether the FL-polymers were interacting with the TCR independent of MHC Class II molecules, we tested the transfectants for the expression of MHC Class II gene products by Northern analysis (Figure 4). We incubated a series of transfected cells with the polymer $FL_{1000}Dex_{2500}$ (Table 2) and PMA for 24 hours as we described earlier (Fig. 3 *B*). RNA was prepared from those cells and from control cell lines such as Jurkat, 31-13, and a EBV transformed autologous B cell line (RSEBV). The RNA was separated on a 1% agarose gel, and transferred to nitrocellulose, where it was probed with a combination of three MHC Class II β cDNAs from DP, DQ, and DR under normal stringency conditions at 42°C (50% formamide and 5× SSC hybridization buffer; 35). Only the RNA from the EBV transformed B cells had detectable MHC Class II mRNA expression (Fig. 4, lane *8*), whereas none of the Jurkat derivatives including those which expressed the $\alpha_{FL}\beta_{FL}$ TCR had detectable MHC mRNA expression (Figure 4, lanes *1–7* and *9*). Other Northern blots probed with each MHC Class II cDNA separately show an equivalent pattern of expression in the EBV transformed B cells as well as the Jurkat derivatives (Data not Shown). All of these Jurkat derivative cell lines express MHC Class I RNA in abundance (Data Not Shown), although Jurkat does not have any detectable CD8 gene expression (Figure 1 *E*, lane *8*). These molecular analyses provide strong evidence that the interaction of the FL antigen with the TCR is independent of the involvement of MHC Class II gene products. We provide further evidence below using a system of FL substituted Sepharose beads.

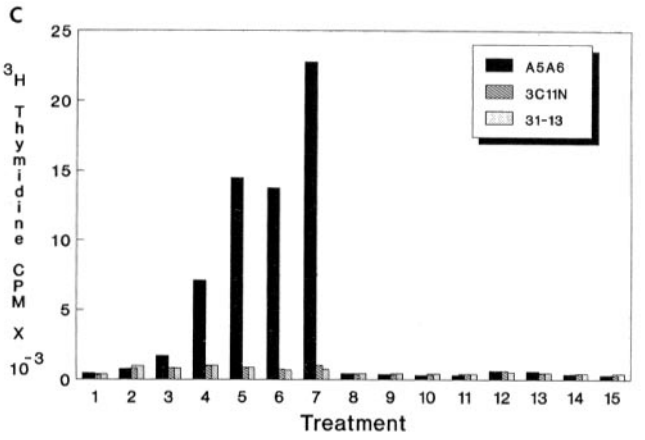
FL-Substituted Sepharose Beads Activate $\alpha_{FL}\beta_{FL}$ Transfected T Cells without PMA. It was apparent that in order to develop a system where the FL antigen directly interacted with T cells, in the absence of other stimuli or signals, it would be necessary to eliminate the need for PMA. It was previously shown that Sepharose 4B beads substituted with FITC could stimulate the T cell clone RFL 3.8 to secrete IL-2 in the absence of accessory cells or exogenous IL-2 (27). We reacted Sepharose 4B beads with FITC (FL Beads), incubated them with transfected cell lines for 24 h, and assayed supernatants for IL-2. The results are shown in Fig. 5 *B*. The FL Beads were fully capable of activating the $\alpha_{FL}\beta_{FL}$ cell line without

A

Fluorescein Derivatives Conjugated To CNBr Activated Sepharose 4B

Structure	Chemical Name	Spacer H H R-N-$(CH_2)_6$-N-R	Location
	FL-6-isothiocyanate	yes	Figure 4C,2-3
	FL-5-isothiocyanate	yes	",4-5
	5((5-aminopentyl)thioureidyl)FL	no	",6-7
	5((2-aminoethyl)thioureidyl)FL	no	",8-9
	5((((2-aminoethyl)thio)acetyl) amino)FL	no	",10-11
	5(aminoacetamido)FL	no	",12-13
	5-(4,6-dichlorotriazinyl)aminoFL	yes	",14-15
	4'((aminoacetamido)methyl)FL	no	Figure 4D,16-17
	tetramethylrhodamine-5-(and-6)-isothiocyanate	yes	",18-19
	5,(and-6)carboxynaphoFL succinimidyl ester	yes	",20-21
	eosin-5-isothiocyanate	yes	",22-23
	erythrosin-5-isothiocyanate	yes	",24-25
	rhodamine-5-(and-6)-isothiocyanate	yes	",26-27





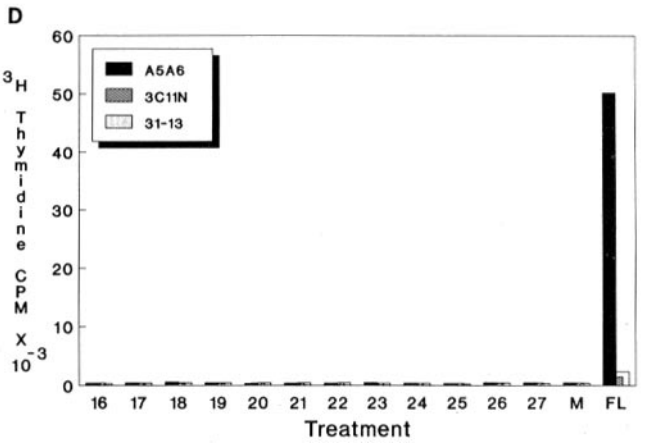


Figure 5. FL-derivatives: activation properties on $\alpha_{FL}\beta_{FL}$ T cell transfectants. (*A*) Summary of FL-derivatives used in the analysis. The compounds shown in (*A*) were reacted with CNBR Activated Sepharose 4B with (Spacer) or without previous reaction with 1,6, diaminohexane. In (*C* and *D*) all of the even numbers correspond to 1 mM of FL derivitization, and the odd numbers to 8 mM. In (*C*) all of the compounds differ from FITC in the "bottom" ring, while in (*D*) the "top" ring is substituted. (*B*) The indicated cell lines were mixed with FITC substituted Sepharose 4B beads containing the 1,6 diaminohexane spacer group. Other treatments such as PMA (P), Medium, or FL-Polymer+P are for comparison purpose, and were done simultaneously with the other treatments. Refer to Fig. 3 *A* and Materials and Methods for details of the methods. (*C*) The indicated cell lines were treated with derivatized Sepharose 4B beads as shown in (*A*). The first series of bars represents results obtained with ethanolamine-substituted Sepharose 4B beads. (*D*) same as (*C*), except that the last series of bars represent results obtained with cells cultured with medium (M), or FL-Polymer (FL).

PMA, and to a level that is comparable to the FL-Polymer and PMA treatment of the same cell line (Fig. 5 *B*). The degree of activation in most experiments is dependent on the concentration of FL on the bead, as exemplified in two separate cases where the Sepharose beads are reacted with 8mM FL derivative, and they are almost twice as effective as those reacted with 1 mM FL (compare lanes *4* and *6* with *5* and 7 in Fig. 5 *C*). We have also found that FITC-derivatized APC (an autologous EBV-transformed B cell line) are able to specifically stimulate $\alpha_{FL}\beta_{FL}$ T cell transfectants such as E3E4, but not control cell lines such as 31-13 or 3C11N (data not shown). Therefore, the $\alpha_{FL}\beta_{FL}$ transfectants are able to recognize FL in the context of an APC, although there is no mandatory requirement for APC participation in the recognition complex of TCR+FL.

Fine Specificity of the $\alpha_{FL}\beta_{FL}$ TCR for FL Derivatives. We took advantage of the ability of FL derivatized Sepharose beads to stimulate the $\alpha_{FL}\beta_{FL}$ transfectants to explore further the specificity of the TCR for derivatives of FL (Figure 5 *A*). We coupled various derivatives of FL to Sepharose directly using available primary amines, or reacted the isothiocyanate forms of the FL derivatives to 1,6 diaminohexane substituted Sepharose (Figure 5 *A*). We then treated three different cell lines with the derivatized beads for 24 hours, and measured IL-2 in the supernatants using a CTLL assay. The results are presented in Figures 5 *C* and *D*. We find a remarkable specificity of the TCR such that the mere alteration of the "bottom" ring substituent from 5' to the 6' position renders the compound almost completely unable to induce T cell activation. More radical substitutions to the triple ring obliterate any activity as shown in Figure 5 *D*. Only FL-Cad with its relatively long pentyl group was effective in activation, although the ethyl form (Figure 5 *C*, treatment 8, 9) was ineffective. In an effort to predict the importance of the N-C = S linkage to activity, we used derivatives which had an oxygen substituted for the sulphur (Figure 5 *C*, treatment 10–13). To our surprise both compounds were completely inert to activation of the $\alpha_{FL}\beta_{FL}$ cells. These results show the exquisite specificity of the TCR for a particular antigen conformation and structure, and illustrate the complexity of studying interactions of more complex soluble antigens with TCRs.

Discussion

Recognition of antigen by T cells has been shown by a variety of methods to be restricted by the gene products of the MHC (see Introduction and reviewed in 44 and 14). It is generally accepted that a particular antigen is recognized by T cells only if processed fragments of the antigen bound to an MHC molecule are recognized by the TCR. Several studies have addressed the question as to whether a hapten can interact with TCR without MHC involvement. The hapten HSAB coupled to polyacrylamide beads was able directly to stimulate a murine T cell hybridoma, and the response could be effectively competed by soluble monomeric hapten (24). Other studies showed that the haptens TNP (47) and p-ABA (23) conjugated to a variety of proteins were capable of binding and activating specific T cell clones. The murine $V_\alpha 3$ gene segment in combination with different $V\beta$ gene segments was found to be capable of imparting specificity for p-ABA substituted proteins (48). Finally, in related studies using a chimeric immunoglobulin-TCR protein, it has been elegantly shown that the haptenic antigen coupled to Sepharose will activate the transfectant to make IL-2 (49).

Although the studies cited above indicate that haptens and hapten-protein conjugates can interact with TCR, the role of MHC molecules in these interactions has not been fully clarified. To address this concern, we have analyzed FL-TCR interactions in a system devoid of the relevant MHC molecules. Several lines of evidence suggest that FL can interact with the $\alpha_{FL}\beta_{FL}$ TCR from a $CD4^+$ FL-specific T cell clone in a functionally significant manner even in the absence of Class II MHC gene products. First, $\alpha_{FL}\beta_{FL}$ transfectants that do not express detectable Class II MHC mRNA, bind FL-Polymers in a highly specific manner. Second, in the presence of PMA, FL-Polymers activate $\alpha_{FL}\beta_{FL}$ transfectants, but not other T cells to produce IL-2. Cross-linking of TCR is important in this response, since monomeric FL is incapable of activation, although it can serve to a limited extent as a competitive inhibitor of FL-Polymer binding (27 and unpublished results). Third, FL derivatized Sepharose beads activate the $\alpha_{FL}\beta_{FL}$ transfectants in a manner that shows exquisite antigen specificity (see below). This effect is mediated by the interaction of a matrix of FL groups with multiple TCRs on the T cell. Although physiologic antigen recognition clearly involves MHC presentation of processed antigen, our results conclusively demonstrate that hapten specific T cells can be activated by appropriate antigen-TCR interactions in the complete absence of the relevant class of MHC molecules.

Although the Jurkat transfectants described here express class I MHC gene products, a previous analysis with blocking antibodies indicate that class I molecules expressed by the T cell have no direct role in FL binding or in subsequent activation events. Studies with FL-specific T cell clones have shown that binding of FL polymers is completely unaffected by the presence of saturating concentrations of anti-class I antibodies (27). Even in the case of the FL-specific clones RFL 3.45 and RFL 1.65 which are $CD8^+$ and class I restricted, blocking experiments with the anti-class I antibody W6/32 showed absolutely no inhibitory effect. Thus, class I is not required for FL binding to the T cell receptor on these cell lines. This is consistent with the exquisite specificity of FL binding (Figure 5). Only FL-specific clones and not other clones with the same class I MHC genotype bind FL (27). Furthermore, as shown here, only those transfectants with an appropriate combination of T cell receptor α and β chains show FL binding even though all express the same complement of class I MHC molecules (Figure 2). The class I independence of the antigen binding of FL-specific T cells is further supported by the ability of solubilized RFL 3.8 T cell receptor molecules to bind to FL columns which do not significantly bind class I MHC molecules (27).

We have taken advantage of the direct interaction of antigen with TCR to study the fine specificity of the TCR

for derivatives of FL. Our main conclusions are that the $\alpha_{FL}\beta_{FL}$ TCR is sensitive to any alteration of the "top" (triple ring) or "bottom" (single ring) of the FL structure (Figures 4 *A–D*). The exchange of the isothiocyanate group from the 5′ to the 6′ position effectively disables the FL moiety from being an activator (Figure 5 *C, 2–3*). Additionally, the substitution of an amide (N-C-C) for a thiourea (N-C-N) linkage also eliminates recognition of the compound, with the caveat that the aminothioacetyl derivative of FL also differs slightly from the other methylene containing FL compounds in the spacer region (compare Figure 5 *C 4–9* and *10–13*). The relatively minor changes in the 5′ bottom ring substituent (Figure 5 *C, 10–11*) completely abolishes the activating function. These data suggest that the single atomic substitution of an oxygen for a sulphur prevents the molecule from serving as an effective hapten for the $\alpha_{FL}\beta_{FL}$ TCR under study.

It was not clear from examining the FL derivatives such as 5(aminoacetamido)FL and FL-Cad whether the length of the methylene spacer group (-CH_2-) between the Sepharose bead and the FL moiety was a major factor in the activity of the FL derivative (Figure 5 *C* compare *4–9*). We examined this issue further by comparing the activities of three FL derivatives that were bound to Sepharose by methylene containing spacer groups. FITC was reacted with diaminohexane substituted Sepharose resulting in a 6 carbon spacer group, whereas FL-Cad was directly reacted with the Sepharose producing a 5 carbon spacer. Both of these Sepharose derivatives were very active in stimulating the cell line E3E4 to make IL-2 (Figure 5 *C, 4–5, 6–7*). However, the 5((2-aminoethyl)thioureidyl)FL which contains only a 2 carbon spacer was not effective as an activating agent (Figure 5 *C, 8–9*). The original T cell clone RFL 3.8 was derived by stimulation with FITC derivatized cells on which FL groups were attached primarily to ϵ amino groups of lysine residues in membrane proteins. Therefore, it is not surprising that the TCR recognizes FL beads with 5 and 6 carbon spacers.

We can infer from our results that the $\alpha_{FL}\beta_{FL}$ TCR makes multiple contacts with several different atoms of the FITC molecule. Since FL is a rigid planar molecule there are no conformational isomers. This may explain how small haptens tethered to insoluble matrices are able to stimulate T cells, whereas peptides of sufficient size to contain activating epitopes possess too many conformational isomers that are not recognized by the highly-specific TCR. In fact, the crystal structure of the MHC Class I molecule (50) which shows a cleft that could bind peptides helps explain the ability of MHC-bound peptides to act as T cell activators. The peptide situated in the MHC cleft may be restricted to a limited number conformations, and as a result it could be expected to spend more time in conformations that are recognized by the TCR. Similarly, the same peptide that is recognized by a particular T cell in the context of one Class II MHC molecule, generally shows no activity when bound to APC with a different MHC haplotype. This has been elegantly shown for specific cytochrome C peptides and Class II MHC molecules in the mouse (12). Therefore, without MHC involvement, peptides generally are thought to be incapable of directly stimulating antigen-specific T cells.

One consequence of the requirement for a three way peptide + MHC + TCR interaction is that mutations in any of the components presumably affect the relationships of all the components. The FL system may circumvent this problem by eliminating the need for MHC interactions to obtain recognition of the haptenic antigen and the TCR. Future studies of this antigen: TCR interaction could be conducted on easily transfectable non T cell hosts (51), and a systematic mutagenesis of the TCR V_α and V_β regions will be carried out utilizing the ability of the FL-Polymer to directly interact with the TCR (Buchwalder and Diamond, In Preparation). Creation of a phosphatidyl-inositol linked form of the $\alpha_{FL}\beta_{FL}$ TCR will allow us to prepare a soluble form using phospholipase C in order to study how the FL-Polymer might interact with a soluble TCR (51). These studies will lead to an understanding of how an antigen-specific TCR recognizes its ligand, and which amino acid side chains actually contact the FL molecule.

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References

1. Davis, M.M., Y.-H. Chien, N.R.J. Gascoigne, and S.M. Hedrick. 1984. A Murine T-cell Receptor Gene Complex: Isolation, Structure and Rearrangement. *Nature (Lond.).* 310:387.
2. Wilson, R.K., E. Lai, P. Concannon, R.K. Barth, and L.F. Hood. 1988. Structure, Organization and Polymorphism of Murine and Human T-cell Receptor α and β Chain Gene Families. *Immunol. Rev.* 101:149.
3. Kappler, J.W., R. Kubo, K. Haskins, J. White, and P. Marrack. 1983. The mouse T cell receptor: comparison of MHC-restricted receptors on two T cell hybridomas. *Cell.* 34:727.
4. Kaye, J.A., S. Porcelli, J. Tite, B. Jones, and C.A. Janeway, Jr. 1983. Both a monoclonal antibody and antisera specific for determinants unique to individual cloned helper T cell lines can substitute for antigen and antigen-presenting cells in the activation of T cells. *J. Exp. Med.* 158:836.
5. McIntyre, B.W., and J.P. Allison. 1983. The Mouse T Cell Receptor: Structural Heterogeneity of Molecules of Normal T cells Defined by Xenoantiserum. *Cell.* 34:739.
6. Meuer, S.C., D.A. Cooper, J.C. Hodgdon, R.E. Hussey, K.A. Fitzgerald, S.F. Schlossman, and E.L. Reinherz. 1983. Identification of the receptor for antigen and major histocompatibility complex on human inducer T lymphocytes. *Nature (Lond.).* 222:1239.
7. Samelson, L.E., R.H. Germain, and R.H. Schwartz. 1983. Monoclonal antibodies against the antigen receptor on a cloned T cell hybrid. *Proc. Natl. Acad. Sci. USA.* 80:6972.
8. Benacerraf, B. 1978. A hypothesis to relate the specificity of T lymphocytes and the activity of I-region specific Ir genes in macrophages and B lymphocytes. *J. Immunol.* 120:1809.
9. Zinkernagel, R.M., and P.C. Doherty. 1979. MHC-restricted cytotoxic T cells: Studies on the biological role of polymorphic major transplantation antigens determining T cell restriction-specificity, function and responsiveness. *Adv. Immunol.* 27:51.
10. Babbitt, B., P.M. Allen, G. Matsueda, E. Haber, and E.R. Unanue. 1985. Binding of immunogenic peptides to Ia histocompatibility molecules. *Nature (Lond.).* 317:359.
11. Buus, S., S. Colon, C. Smith, J.H. Freed, C. Miles, and H.M. Grey. 1986. Interaction between a "processed" ovalbumin peptide and Ia molecules. *Proc. Natl. Acad. Sci. USA.* 83:3968.
12. Hedrick, S.M., L.A. Matis, T.T. Hecht, C. Hannum, L.E. Samelson, D.L. Longo, E. Heber-Katz, and R.H. Schwartz. 1982. The fine specificity of antigen and Ia determinant recognition by T cell hybridoma clones specific for pigeon cytochrome c. *Cell.* 30:141.
13. Fink, P., L. Matis, D. McElligot, M. Bookman, and S. Hedrick. 1986. Correlation between T cell specificity and the structure of the antigen receptor. *Nature (Lond.).* 321:219.
14. Saito, T., and T. Germain. 1988. The generation and selection of the T cell repertoire: Insights from studies of the molecular basis of T cell recognition. *Immunol Rev.* 101:81.
15. Clayton, L., M. Sieh, D.A. Pious, and E.L. Reinherz. 1989. Identification of human CD4 residues affecting class II MHC versus HIV-1 gp120 binding. *Nature (Lond.).* 339:548.
16. Lamarre, D., A. Ashkenazi, S. Fleury, D.H. Smith, R.-P. Sekaly, and D.J. Capon. 1989. The MHC-binding and gp120-binding functions of CD4 are separable. *Science (Wash. DC).* 245:743.
17. Norment, A.M., R.D. Salter, P. Parham, V.H. Engelhard, and D.R. Littman. 1988. Cell-cell adhesion mediated by CD8 and MHC class I molecules. *Nature (Lond.).* 336:79.
18. Salter, R.D., R. Benjamin, P.K. Wesley, S.E. Buxton, T.P.J. Garrett, C. Clayberger, A.M. Krensky, A.M. Norment, D.R. Littman, and P. Parham. 1990. A binding site for the T cell co-receptor CD8 on the α_3 domain of HLA-A2. *Nature (Lond.).* 345:41.
19. Dembic, Z., W. Haas, S. Weiss, J. McCubrey, H. Kiefer, H. von Boehmer, and M. Steinmetz. 1986. Transfer of specificity by murine α and β T-cell receptor genes. *Nature (Lond.).* 320:232.
20. Gabert, J., C. Langlet, R. Zamoyska, J.R. Parnes, A.-M. Schmitt-Verhulst, and B. Malissen. 1987. Reconstitution of MHC Class I Specificity by Transfer of the T cell Receptor and Lyt-2 Genes. *Cell.* 50:545.
21. Saito, T., A. Weiss, J. Miller, M.A. Norcross, and R.N. Germain. 1987. Specific antigen-Ia activation of transfected human T cells expressing murine Ti$\alpha\beta$-human T3 receptor complexes. *Nature (Lond.).* 325:125.
22. Carel, S., C. Bron, and C. Corradin. 1983. T cell hybridoma specific for cytochrome c polypeptide-specific antigen binding and interleukin 2 production. *Proc. Natl. Acad. Sci. USA.* 80:4832.
23. Rao, A., W.W.-P. Ko, S.J. Faas, and H. Cantor. 1984. Binding of antigen in the absence of histocompatibility proteins by arsonate-reactive T cell clones. *Cell.* 36:879.
24. Thomas, D.W., and M.J. Solvay. 1986. Direct stimulation of T lymphocytes by antigen-conjugated beads. *J. Immunol.* 87:419.
25. Barnd, L.B., M.S. Lan, R.S. Metzgar, and O.J. Finn. 1989. Specific, major-histocompatibility complex-unrestricted recognition of tumor associated mucins by human cytotoxic T cells. *Proc. Natl. Acad. Sci. USA.* 86:7159.
26. Siliciano, R.F., A.D. Keegan, R.Z. Dintzis, H.M. Dintzis, and H.S. Shin. 1985. The Interaction of Nominal Antigen with T Cell Antigen Receptors. *J. Immunol.* 135:906.
27. Siliciano, R.F., T.J. Hemesath, J.C. Pratt, R. Dintzis, H.M. Dintzis, O. Acuto, H.S. Shin, and E. Reinherz. 1986. Direct evidence for the existence of nominal antigen binding sites on T cell surface Ti heterodimers of MHC restricted T cell clones. *Cell.* 47:161.
28. Dintzis, H.M., R.Z. Dintzis, and B. Vogelstein. 1976. The molecular determinants of immunogenicity: the immunon model of immune response. *Proc. Natl. Acad. Sci. USA.* 73:3671.
29. Dintzis, R.Z., M.H. Middelton, and H.M. Dintzis. 1983. Studies on the immunogenicity and tolerogenicity of T-independent antigens. *J. Immunol.* 131:2196.
30. Gubler, U., and B.J. Hoffman. 1983. A simple and very efficient method for generating cDNA libraries. *Gene.* 25:263.
31. Huynh, T.V., R.A. Young, and R.W. Davis. 1985. Constructing and screening cDNA libraries in λgt10 and λgt11. *In* DNA Cloning: A Practical Approach. D.M. Glover, editor. IRL Press Ltd., Oxford. pp. 49.
32. Royer, H.D., A. Bensussan, O. Acuto, and E.L. Reinherz. 1984. Functional isotypes are not encoded by the constant region genes of the β subunit of the T cell receptor for antigen. *J. Exp. Med.* 160:947.
33. Yoshikai, Y., S.P. Clark, S. Taylor, Y. Sohn, B.I. Wilson, M.D. Minden, and T.W. Mak. 1985. Organization and sequences of the variable, joining and constant region genes of the human T cell receptor α-chain. *Nature (Lond.).* 316:837.
34. Kabat, E.A., T.T. Wu, M. Reid-Miller, H.M. Perry, and K.S. Gottesman. 1987. Sequences of Proteins of Immunological Interest. Fourth Edition. U.S. Department of Health and Human Services, Bethesda, MD. pp. 904.
35. Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
36. Yates, J.L., N. Warren, and B. Sugden. 1985. Stable replica-

tion of plasmids derived from Epstein-Barr virus in various mammalian cells. *Nature (Lond.).* 313:812.
37. Sugden, B., K. Marsh, and J. Yates. 1985. A Vector That Replicates as a Plasmid and Can Be Efficiently Selected in B-Lymphoblasts Transformed by Epstein-Barr Virus. *Mol. Cell. Biol.* 5:410.
38. Reisman, D., and B. Sugden. 1986. Trans Activation of an Epstein-Barr Viral Transcription Enhancer by the Epstein-Barr Viral Nuclear Antigen 1. *Mol. Cell. Biol.* 6:3838.
39. Cann, A.J., Y. Koyanagi, and I.S.Y. Chen. 1988. High efficiency transfection of primary human lymphocytes and studies of gene expression. *Oncogene.* 3:123.
40. Meuer, S.C., S.F. Schlossman, and E.L. Reinherz. 1982. Clonal analysis of human cytotoxic T lymphocytes: T4+ and T8+ effector T cells recognize products of different major histocompatibility complex regions. *Proc. Nat'l. Acad. Sci. USA.* 79:4395.
41. Saito, T., and R.N. Germain. 1989. Marked differences in the efficiency of expression of distinct $\alpha\beta$ T cell receptor heterodimers. *J. Immunol.* 143:3379.
42. Alcover, A., C. Alberini, O. Acuto, L.K. Clayton, C. Transy, G.C. Spagnoli, P. Moingeon, P. Lopez, and E.L. Reinherz. 1988. Interdependence of CD3-Ti and CD2 activation pathways in human T lymphocytes. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1973.
43. Ohashi, P.S., T.W. Mak, P. Van den Elsen, Y. Yanagi, Y. Yoshikai, A.F. Calman, C. Terhorst, J.E. Stobo, and A. Weiss. 1985. Reconstitution of an active surface T3/T-cell antigen receptor by DNA transfer. *Nature (Lond.).* 316:606.
44. Davis, M.M., and P.J. Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. *Nature (Lond.).* 334:395.
45. Wiskocil, R., A. Weis, J. Imboden, R. Kamin-Lewis, and J. Stobo. 1985. Activation of a human T cell line: A two stimulus requirement. *J. Immunol.* 134:1599.
46. Weiss, A., J. Imboden, K. Hardy, B. Manger, C. Terhorst, and J. Stobo. 1986. The role of the T3/antigen receptor complex in T cell activation. *Annu. Rev. Immun.* 4:593.
47. Clayberger, C., R.H. Dekruyff, J Aisenberg, and H. Cantor. 1983. Hapten-reactive Inducer T Cells I. Definition of two classes of Hapten-specific inducer cells. *J. Exp. Med.* 157:1906.
48. Tan, K.-N., B.M. Datlof, J.A. Gilmore, A.C. Kronman, J.H. Lee, A.M. Maxam, and A. Rao. 1988. The T Cell Receptor $V_\alpha 3$ Gene Segment is Associated with Reactivity to p-Azobenzenearsonate. *Cell.* 54:247.
49. Goverman, J., S.M. Gomez, K.D. Segesman, T. Hunkapiller, W.E. Laug, and L. Hood. 1990. Chimeric immunoglobulin-T cell receptor proteins form functional receptors: Implications for T cell receptor complex formation and activation. *Cell.* 60:929.
50. Bjorkman, P.J., M.A. Saper, B. Samroui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature (Lond.).* 329:512.
51. Lin, A.Y., B. Devaux, A. Green, C. Sagerstrom, J.F. Elliott, and M.M. Davis. 1990. Expression of T cell antigen receptor heterodimers in a lipid-linked form. *Science (Wash. DC).* 249:677.