The CD3$^{\varepsilon}$ Cytoplasmic Domain Mediates CD2-induced T Cell Activation

By Frank D. Howard, Philippe Moingeon, Ulrich Moebius, David J. McConkey, Booma Yandava, Tiiu E. Gennert, and Ellis L. Reinherz

From the Laboratory of Immunobiology, Dana-Farber Cancer Institute, and the Departments of *Pathology and †Medicine, Harvard Medical School, Boston, Massachusetts 02115

Summary

CD2-mediated T lymphocyte activation requires surface expression of CD3-Ti, the T cell receptor (TCR) for antigen major histocompatibility complex protein. Given the importance of CD3$^{\varepsilon}$ in TCR signaling, we have directly examined the ability of the CD3$^{\varepsilon}$ cytoplasmic domain to couple CD2 to intracellular signal transduction pathways. A cDNA encoding a chimeric protein consisting of the human CD3$^{\varepsilon}$ cytoplasmic domain (amino acid residues 31-142) fused to the CD8$^{\alpha}$ extracellular and transmembrane domains (amino acid residues 1-187) was transfected into a CD2$^{+}$CD3$^{-}$CD8$^{-}$ variant of the human T cell line Jurkat. The resulting transfectants expressed the CD8$^{\alpha}$/CD3$^{\varepsilon}$ chimeric receptor at the cell surface in the absence of other TCR subunits. Stimulation of these transfectants with anti-T112 + anti-T113 monoclonal antibodies (mAbs) initiated both a prompt cytosolic free calcium ([Ca$^{2+}$]i) rise and protein tyrosine kinase activation. Stimulation with either intact anti-T112 + anti-T113 mAbs or purified F(ab')2 fragments resulted in interleukin 2 (IL-2) secretion. In contrast, control cell lines transfected with a cDNA encoding wild-type CD8$^{\alpha}$, and thus lacking surface expression of the CD3$^{\varepsilon}$ cytoplasmic domain, failed to show any [Ca$^{2+}$]i rise, protein tyrosine kinase activation, or IL-2 secretion after identical stimulation. These data directly establish the CD3$^{\varepsilon}$ cytoplasmic domain as a necessary and sufficient component of the CD3-Ti complex involved in T lymphocyte activation through CD2. Moreover, they show that CD2 signaling can function in the absence of Fc receptors.

Materials and Methods

Cell Lines. J77 is a CD2$^{+}$CD3$^{+}$Ti$^{+}$CD8$^{-}$CD16$^{-}$ derivative of the leukemic human T cell line Jurkat. 31-13 is a CD2$^{+}$CD3$^{+}$Ti$^{-}$ mutant of J77 lacking a functional Ti$^{\delta}$ subunit (8). These lines were maintained at 37°C in culture medium (RPMI 1640 + 10% FCS, 1 mM sodium pyruvate, 2 mM l-glutamine, 50 U/ml penicillin-streptomycin) under 5% CO$_2$. WT-6, derived by transfection of 31-13 with J77 T$^{\alpha}$ cDNA, thereby restoring surface CD3$^{+}$Ti$^{+}$ expression (8), was maintained in culture medium supplemented with 0.5 mg/ml G418 (Gibco/BRL, Gaithersburg, MD).

Antibodies. The specificities, designations, and isotypes of the murine mAbs used in this study are as follows: anti-CD2: anti-T112, 3T4-SB5, IgG2a; anti-T113: 1Old2-4C1, IgG2; anti-T11$: 1Mono2A6, IgG3; anti-CD3: 2Ad2A2, IgM, or RW28C8, IgG1; anti-CD4: 19ThySD7, IgG2; anti-CD8: 21Thy2D3, IgG1. N23 is a rabbit anti-human CD3$^{\varepsilon}$/antiserum raised against the human CD3$^{\varepsilon}$/COOH-terminal peptide CDTYDALHMQALPPR conjugated to BSA using sulfo-MBS (Pierce Chemical Co., Rockford, IL) according to the supplier's directions and shown to immunoprecipitate and Western blot human CD3$^{\varepsilon}$. 4G10 antiphosphotyrosine murine mAb was kindly provided by Dr. Brian Drucker (DFCI, Boston, MA) (15). Purified anti-T112 and anti-T113 F(ab')2 fragments were prepared as described (16).
Chimera Generation. Using PCR techniques, the complete coding region of human CD8\(\alpha\) was amplified from clone pL2-M (17) (kindly provided by Dr. Jane Parnes, Stanford University, Stanford, CA) using oligonucleotides containing a 5' BglII recognition sequence (CD8\(\alpha\) 5' sense oligonucleotide: nucleotides 88-108 of reference 17, 5'GCAGAGGATCTTTGCGGCTAATCTGAGTGCACACTCCTGTTGTTGCGAGCTAAAGGGG3'; CD8\(\alpha\) 3' antisense oligonucleotide: nucleotides 773-795 of reference 17, 5'CGGGAATCCTTTAGGTTGCAGTAAAGGG3'). The two overlapping PCR fragments encompassing these regions amplified from CD8\(\alpha\) (clone pl2M) and CD3\(\varepsilon\) (clone pGEM3\(\varepsilon\)) (19), kindly provided by Drs. Allan Weissman and Richard Klauber, National Institutes of Health, Bethesda, MD), respectively (CD8\(\alpha\) chimera 5' sense oligonucleotide identical to CD8\(\alpha\) 5' sense oligonucleotide listed above; CD8\(\alpha\) 3' overlap sense oligonucleotide: 5'GCCTGCTGCGAGGATCTTTGCGGCTAATCTGAGTGCACACTCCTGTTGTTGCGAGCTAAAGGGG3'; CD8\(\alpha\) 3' overlap sense oligonucleotide: 5'GTTGATATCCCTTTAGGTTGCAGTAAAGGG3'; CD3\(\varepsilon\) antisense oligonucleotide nucleotides 545-566 from reference 19; 5'CGGGAATTCTTTAGGTTGCAGTAAAGGG3'; CD8\(\alpha\)/CD3\(\varepsilon\) overlap sense nucleotides 577-599 of reference 17, 5'CGGGAATTCTTTAGGTTGCAGTAAAGGG3'; CD8\(\alpha\)/CD3\(\varepsilon\) overlap antisense oligonucleotide nucleotides 773-795 of reference 17, 5'CGGGAATCCTTTAGGTTGCAGTAAAGGG3'). The two overlapping PCR fragments were annealed and filled in using the Klenow fragment of DNA polymerase, and 10% of the products from this reaction were subjected to PCR amplification with CD8\(\alpha\) 5' sense and CD3\(\varepsilon\) 3' antisense oligonucleotides to yield the chimeric CD8\(\alpha\)/CD3\(\varepsilon\) PCR fragment. After gel purification and BglII digestion, the fragment was subcloned into the BamHI site of the expression vector pPink2 (18) creating a construct termed pCD8\(\alpha\)/Pink2 for use in transfection (see below). Similarly, a chimeric cDNA encoding the NH2-terminal 187 amino acids of CD8\(\alpha\) fused to the coding region of human CD3\(\varepsilon\) was amplified from clone pL2-M (18) (kindly provided by Drs. Allan Weissman and Richard Klauber, National Institutes of Health, Bethesda, MD), respectively (CD8\(\alpha\) chimera 5' sense oligonucleotide identical to CD8\(\alpha\) 5' sense oligonucleotide listed above; CD8\(\alpha\) 3' overlap sense oligonucleotide: 5'GCCTGCTGCGAGGATCTTTGCGGCTAATCTGAGTGCACACTCCTGTTGTTGCGAGCTAAAGGGG3'; CD8\(\alpha\) 3' overlap sense oligonucleotide: 5'GTTGATATCCCTTTAGGTTGCAGTAAAGGG3'; CD3\(\varepsilon\) antisense oligonucleotide nucleotides 545-566 from reference 19; 5'CGGGAATTCTTTAGGTTGCAGTAAAGGG3'; CD8\(\alpha\)/CD3\(\varepsilon\) overlap sense nucleotides 577-599 of reference 17, 5'CGGGAATTCTTTAGGTTGCAGTAAAGGG3'; CD8\(\alpha\)/CD3\(\varepsilon\) overlap antisense oligonucleotide nucleotides 773-795 of reference 17, 5'CGGGAATCCTTTAGGTTGCAGTAAAGGG3'). The two overlapping PCR fragments were annealed and filled in using the Klenow fragment of DNA polymerase, and 10% of the products from this reaction were subjected to PCR amplification with CD8\(\alpha\) 5' sense and CD3\(\varepsilon\) 3' antisense oligonucleotides to yield the chimeric CD8\(\alpha\)/CD3\(\varepsilon\) PCR fragment. After gel purification and BglII digestion, the fragment was subcloned into the BamHI site of pPink2 resulting in a construct termed pCD8\(\alpha\)/CD3\(\varepsilon\)/Pink2. Identity of the resulting plasmids was verified by restriction enzyme analysis and DNA sequencing.

Transfections and Selection. pCD8\(\alpha\)/CD3\(\varepsilon\)/Pink2 and pCD8\(\alpha\)/Pink2 were each transfected into 31-13 cells by electroporation using a cell porator system (Gibco/BRL) with a capacitance set at 330 \(\mu\)F. Transfectants were selected in culture medium supplemented with 1.0 mg/ml G418. Resistant clones were further selected for staining with 1.0 mg/ml G418-free culture medium containing 2 \(\mu\)g/ml of the acetoxyethyl ester of indo-1 (Molecular Probes, Eugene, OR) for 45 min at 37\(^\circ\)C. Subsequently, cells were pelleted and resuspended in 2 ml culture medium. Fluorescence measurements were made using a fluorimeter (SFM25; Kontron, Zürich, Switzerland) with excitation and emission wavelengths of 350 and 405 nm, respectively. Baseline fluorescence was monitored for 3 min, after which a 1:200 dilution of antibody was added and fluorescence monitored for 15 min. The cells were kept in suspension by constant stirring. At the end of each analysis, maximal fluorescence was determined by adding Triton X-100 to 0.05% and minimal fluorescence determined after addition of EGTA to 2.5 mM. [Ca\(^{2+}\)] was calculated as described (21).

Detection of IL-2 Production. 10\(^{6}\) cells were plated in 96-well round-bottomed plates (final volume, 0.2 ml) and stimulated at 37\(^\circ\)C for 24 h in the presence of 5 ng/ml PMA with either anti-CD3 (2D\(\alpha\)2A2), anti-T\(\varepsilon\)I1 (10D4-2C1) plus anti-T\(\varepsilon\)I1 (1Mono2A6), anti-CD8 (21Thy2D3), or calcium ionophore (A23187) at 1 \(\mu\)g/ml final concentration (Sigma Chemical Co.). All mAbs were used as either as ascites at saturating concentration (1:200 final) or in purified form at 10 \(\mu\)g/ml final concentration. Serial dilutions of culture supernatants were then tested for their capacity to support proliferation of 10\(^{4}\) CTLb20 cells as described (22).

Results and Discussion

Transfection of the Jurkat CD3\(\varepsilon\) mutant line 31-13 resulted in 12 independently derived surface CD8\(\varepsilon\)-expressing clones for each cDNA denoted 31-13.o\(\varepsilon\) for plasmid pCD8\(\alpha\)/CD3\(\varepsilon\)/Pink2, respectively. Flow cytometric analysis of representative clones for each construct are shown in Fig. 1. Whereas J77, 31-13, and WT-6 (a 31-13 transfectant in which CD3-T\(\varepsilon\)I surface expression has been restored after transfection of a wild-type T\(\beta\) cDNA [8]) express detectable CD8\(\varepsilon\), 31-13 transfectants containing either full-length CD8\(\alpha\) cDNA (31-13.o\(\varepsilon\)2) or chimeric CD8\(\alpha\)/CD3\(\varepsilon\) cDNA (31-13.o\(\varepsilon\)6, 31-13.o\(\varepsilon\)11) expressed readily detectable CD8\(\varepsilon\) at the surface. The expression level of CD8\(\varepsilon\) was reproducibly greater in the native CD8\(\alpha\)-containing lines as compared with clones expressing CD8\(\alpha\)/CD3\(\varepsilon\) chimera.

1 Abbreviation used in this paper: [Ca\(^{2+}\)]\(_{i}\), cytosolic free calcium.
CD2 CD3 CD8

J77
31-13
WT6
31-13.a2
31-13.a3

31-13.a6

Cell Number

Log Fluorescence Intensity

Figure 1. Surface phenotype of Jurkat derivatives and transfectants. Immunofluorescence analysis of Jurkat derivatives J77, 31-13 (CD3-/Ti-), WT6 (CD3+/Ti+), 31-13.a2 (CD3-/Ti-CD8α+), 31-13.a3 (CD3-/Ti-CD8α/CD3δ+), and 31-13.a6 (CD3-/Ti-CD8α/CD3δ+). Histograms display reactivity with the indicated murine anti-human mAb (thick line) as compared with second step alone (thin line).

Figure 2. Biochemical characterization of 31-13 transfectants. Surface iodinated cells were lysed in digitonin containing lysis buffer and immunoprecipitated with the indicated antibody-conjugated beads (anti-CD2, anti-CD3, and anti-CD8) or antibody-coated protein A-Sepharose (anti-CD4, rabbit anti-CD3δ). Immunoprecipitates were dissolved in Laemmli SDS sample buffer, and analyzed by SDS-PAGE (12.5% acrylamide) and subsequent autoradiography. Each lane contains material from ~10^7 cells. (A) Analysis of CD8α (31-13.a2) and CD8α/CD3δ (31-13.a6)-expressing transfectants under nonreducing conditions after immunoprecipitation with anti-CD2, anti-CD3, and anti-CD4. (B) Analysis of CD8α (31-13.a2) and CD8α/CD3δ (31-13.a3) transfectants under reducing and nonreducing conditions after immunoprecipitation with anti-CD8 or rabbit anti-CD3δ (antiserum N23) as indicated. (C) Western blot analysis of CD8α (31-13.a4) and CD8α/CD3δ (31-13.a4)-expressing transfectants. Anti-CD8 immunoprecipitates of 31-13.a4 and 31-13.a5 were transferred to nitrocellulose after SDS-PAGE and probed with the rabbit anti-CD3δ antiserum N23 as described in Materials and Methods.
bands on one-dimensional analysis with anti-CD3ζ under reducing conditions (Fig. 2 B; and data not shown), we conclude that the higher molecular mass species seen under non-reducing conditions represent homo-multimers.

The ability of the chimeric protein to participate in transducing T cell activation signals was assessed by monitoring [Ca^{2+}] mobilization as a function of surface receptor cross-linking. As shown for WT-6 (CD2^+CD3^-CD8^-), addition of an anti-CD3 antibody or a mitogenic combination of anti-CD2 antibodies results in a prompt rise in intracellular calcium (Fig. 3, B and C). As expected, anti-CD8 did not elicit a calcium rise in these cells (Fig. 3 A), even when rabbit anti-mouse Ig was added as a second step (data not shown). Moreover, stimulation of the CD2^-CD3^- parental line 31-13 with anti-CD2, CD3, or CD8 mAbs failed to mobilize calcium (Fig. 3, D, E, and F). No calcium rise was observed in cell lines expressing the full-length CD8α protein after stimulation with anti-CD8 (Fig. 3, G and J). In contrast, after anti-CD8 treatment of CD8α/CD3ζ-expressing clones, a prompt rise in intracellular calcium was detectable within 3 min (Fig. 3, M and P). These results demonstrate the importance of the CD3ζ subunit in mediating signaling and are entirely consistent with previous observations (13-15).

We next determined whether the CD8α/CD3ζ structure could functionally substitute for the entire CD3-Ti complex and allow T cell activation through CD2. As shown in Fig. 3, CD2 crosslinking with anti-T112 plus anti-T113 resulted in a prompt increase in [Ca^{2+}] in the case of CD8α/CD3ζ chimeras-containing clones (shown for 31-13.αζζ; Fig. 3, N and Q), similar to that seen in WT-6 (B). In contrast, no calcium rise was detected in transfectants expressing full-length CD8α (31-13.αζ2; Fig. 3, H and K). The lack of detectable calcium mobilization in response to anti-CD3 in 31-13 and all CD8α and CD8α/CD3ζ transfectants as well as the CD3^- parental line (Fig. 3, F, I, L, O, and R) is consistent with the absence of surface CD3. Crosslinking with anti-CD4 failed to elicit a detectable calcium rise in any

### Table 1. IL-2 Production by CD8α/CD3ζ Chimera-expressing Cell Lines

| Exp. | Cells | Media | Anti-CD3 | Anti-CD8 | Anti-T11ζ,ζ | Ca ionophore (anti-T11ζ,ζ) | F(ab')2 |
|------|-------|-------|---------|----------|-------------|-----------------------------|--------|
|      |       |       |         |          |             |                             | U/ml   |
| 1    | WT-6  | <2    | 32      | <2       | 512         | 256                         |        |
|      | 31-13 | <2    | <2      | <2       | <2          | 128                         |        |
|      | 31-13.αζζ | <2    | <2      | 32       | 32          | 128                         |        |
|      | 31-13.αζζ1 | <2     | 64      | 64       | 128         |                             |        |
|      | 31-13.αζ2 | 2      | <2      | 2        | 2           | 256                         |        |
|      | WT-6  | <2    | <2      | 256      | 128         | 128                         | 32     |
|      | 31-13.αζζ | 2      | 64      | 128      | 32          |                             |        |
|      | 31-13.αζ2 | 2      | <2      | 2        | <2          |                             |        |

Cells were incubated for 24 h with various stimuli in the presence of 5 ng/ml PMA (final concentration). For Exp. 2, all antibodies and F(ab')2 fragments were used at 10 μg/ml final concentration. Supernatants were harvested and tested in duplicate in serial dilution for their capacity to support the growth of the IL-2-dependent murine T cell line CTLL20. Results are expressed in arbitrary U/ml of IL-2 secreted. The last dilution able to maintain the viability of 10,000 CTLL-20 cells was defined as containing 1 arbitrary U/ml. Titration experiments performed in parallel with rIL-2 (Biogen, Cambridge, MA) revealed that 1 arbitrary U is equivalent to ∼1.2 U of rIL-2. The data shown are representative of three (Exp. 1) or two (Exp. 2) independent experiments.
Figure 4. Protein tyrosine kinase activation of a CD8α/CD3ζ-expressing 31-13 transfectant by anti-CD2 or anti-CD8. Aliquots of 10⁷ 31-13.ot~6 cells were stimulated with the indicated antibodies at a 1:100 dilution for various times in minutes as shown. 10% of the postnuclear lysate from each sample was directly subjected to SDS-PAGE (7.5-17.5% acrylamide), while the remaining 90% was immunoprecipitated with rabbit anti-CD3~" antiserum N23, and then subjected to SDS-PAGE (7.5-17.5% acrylamide). After transfer to nitrocellulose, all samples were probed with an- tiphosphotyrosine mAb 4G10 and developed as described in Materials and Methods. (a) Tyrosine phosphorylation of proteins in total postnuclear lysates of 31-13.ot~6 cells stimulated with anti-CD2, anti-CD3, or anti-CD8 (anti-T112 + anti-T113) as a function of time. Bands appearing as a result of stimulation are denoted pp170, pp110, pp100, pp72, and pp34. IgH and IgL denote antibody heavy chain and light chain, respectively. (b) Tyrosine phosphorylation of CD8α/CD3ζ chimeric protein from anti-CD3ζ immunoprecipitates of 31-13.ot~6 cells stimulated as in a. The CD8α/CD3ζ chimera is denoted pp34.

We further investigated the ability of the chimeric CD8α/CD3ζ to reconstitute CD2-stimulated IL-2 production in the absence of surface CD3/Ti. As expected, stimulation of CD8α/CD3ζ chimera-expressing cells with anti-CD8 resulted in IL-2 production (Table 1, Exp. 1). The magnitude of the IL-2 response from CD8α/CD3ζ chimera-expressing lines after CD8 triggering was consistently found to be quantitatively similar to the levels of IL-2 production from CD3/Ti⁺ line WT-6 stimulated with anti-CD3. More importantly, stimulation of CD8α/CD3ζ chimera-expressing TCR⁺ clones, of which 31-13.αζ6 and 31-13.αζ11 are representative, with anti-T112 plus anti-T113 for 24 h, resulted in significant levels of IL-2 production. As previously reported, stimulation of WT-6 through CD2 resulted in an IL-2 response exceeding that of anti-CD3 stimulation by an order of magnitude (8). Interestingly, CD2 stimulation of CD8α/CD3ζ chimera-expressing TCR⁺ clones produced levels of IL-2 comparable in magnitude with CD8 triggering of these cells, but clearly less than the IL-2 response of CD2-stimulated WT-6 (Table 1, Exp. 1). CD2 stimulation of WT6 or 31-13.αζ6 using purified anti-T112 plus anti-T113 (ab')₂ fragments resulted in levels of IL-2 production comparable with levels seen using intact IgG (Table 1, Exp. 2). As expected, the CD3⁻ line 31-13 and transfectants expressing only full-length CD8α, of which 31-13.α2 is representative, failed to produce detectable amounts of IL-2 to any of the above stimuli. Nevertheless, since a combination of PMA and calcium ionophore induced IL-2 production from each cell line tested, lack of IL-2 production from 31-13 or 31-13.α2 is not a consequence of an intrinsic defect in IL-2 gene induction (Table 1, Exp. 1). The ability of purified anti-T112 plus anti-T113 (ab')₂ fragments to elicit IL-2 production in the CD8α/CD3ζ chimera-expressing clone excludes a role for Fc receptors in mediating this effect.

To examine the role of the CD8α/CD3ζ chimera in CD2-stimulated protein tyrosine kinase activation, we compared the patterns of tyrosine phosphorylated proteins after stimulation through CD2 or the chimeric receptor. Western blot analysis of 31-13.αζ6 total cellular lysates using an antiphosphotyrosine mAb disclosed a number of substrates that became tyrosine phosphorylated as a result of either anti-CD2 or anti-CD8 stimulation when compared with the zero time points or stimulation with anti-CD3 (Fig. 4a, lanes 1-4 and 5-13). Interestingly, several substrates were phosphorylated in response to either anti-CD2 or anti-CD8 (denoted pp170, pp110, pp100, pp72), whereas at least one substrate (pp110) was evident only after CD2 stimulation (Figure 4a, compare lanes 4, 7, 10, and 13 with lanes 2, 5, 8, and 11). In addition, the appearance of new bands in the 34-kD range suggested that the chimeric receptor was tyrosine phosphorylated as a result of either anti-CD2 or anti-CD8 stimulation when compared with the zero time points or stimulation with anti-CD3 (Fig. 4a, lanes 1-4 and 5-13). To confirm this, anti-CD3ζ immunoprecipitates of part of the material analyzed in Fig. 4a were subjected to SDS-PAGE and Western blotting using antiphosphotyrosine mAb. This analysis clearly demonstrates that the chimeric receptor (denoted pp34) is phosphorylated as a result of either anti-CD8 or CD2 stimulation (Fig. 4b, lanes 5, 8, and 11) or anti-CD2 stimulation (Fig. 4b, lanes 7, 10,
and 12). It should be noted that the time to maximal CD8α/CD3ζ phosphorylation was much shorter for CD8 stimulation (≈1 min) than for CD2-mediated activation (≈15 min) (Fig. 4b, lanes 4 and 13; and data not shown). The basis for this kinetic difference remains to be determined. As expected, stimulation of cell line 31.12.a2 with CD2, CD3 or CD8 failed to yield any specific tyrosine phosphorylated protein bands in Western blot analysis of either total cellular lysates or anti-CD3ζ immunoprecipitates (data not shown).

That the cytoplasmic domain of CD3ζ can function in lieu of the TCR complex (Tia-β/CD3γδε[ζ-ζ' or ζ-η]) in facilitating T cell activation through CD2 suggests that CD3ζ is a critical TCR component in T cells necessary for coupling CD2 to cytoplasmic second messenger pathways. This conclusion is particularly noteworthy given that in NK cells, in contrast to T cells, CD2 signaling activates second messenger pathways in the absence of TCR (23). Interestingly, NK cells express cell surface CD3ζ as a component of a molecular complex with the low affinity IgG Fc receptor CD16 (FcyRIII) (24). This observation suggests that in NK cells CD3ζ may also play a central role in CD2-mediated triggering. Consistent with this notion, we have recently shown that transfection of a CD3ζ-CD2ζ Jurkat line with the transmembrane form of CD16 results in surface expression of CD16 in association with endogenous CD3ζ homodimers and restores CD2-mediated activation (16). While these results further underscore the importance of CD3ζ in mediating CD2 signaling, we cannot exclude a functional role for other components of the TCR (e.g., CD3γδε) in this process. In fact, the quantitative difference in IL-2 production by CD2-stimulated CD8α/CD3ζ chimera-expressing lines as compared with TCR-expressing lines may reflect the participation of other TCR components in the CD2 signaling pathway. Although previous work has documented a role for Fc receptors in anti-CD2 mAb-mediated signaling under some experimental conditions (25, 26), the present findings with the FcRζ- cell line Jurkat using purified F(ab')2 fragments show that CD2 triggering can function independently of FcR subunits.

The biochemical basis of the functional dependence of CD2 signaling on CD3ζ is presently unknown. It is clear, however, that the cytoplasmic domains of both CD2 and CD3ζ are required for this functional interaction (16, 27), which implies that the relevant biochemical components are intracellular structures. The fact that T cell activation through the PI-linked surface structure Thy-1 has been shown to require cell surface CD3ζ (10) makes a direct physical association between this extracellular structure and CD3ζ unlikely. Similarly, the interaction between CD2 and CD3ζ, if not direct, may be indirect and/or transient and involve as yet unidentified cellular constituents. The ability of a single chain chimeric receptor to link accessory molecules to second messenger pathways should further facilitate molecular dissection of biochemical components and interactions important in mediating T cell activation through CD2 and other accessory structures.

We are grateful to Drs. Linda K. Clayton and Gilles Salles for helpful comments, to Amanda Mildonian and Sheena Abraham for expert technical assistance, to Dr. Jane Parnes for supplying the CD8α cDNA clone, to Drs. Allan Weissman and Richard Klausner for the CD3ζ cDNA clone, and to Dr. Brian Druker for 4G10 mAb.

This work was supported by National Institutes of Health grant AI-19807, and a Howard Hughes Medical Institute Postdoctoral Fellowship for Physicians award to F. D. Howard.

Address correspondence to Frank D. Howard, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.

Received for publication 21 November 1991 and in revised form 6 January 1992.

References

1. Alcover, A., D. Ramarli, N.E. Richardson, H.-C. Chang, and E.L. Reinherz. 1987. Functional and molecular aspects of human T lymphocyte activation via T3-Ti and Ti1 pathways. ImmunoL Rev. 95:5.

2. Gunter, K.C., T.R. Malek, and E.M. Shevach. 1984. T cell activating properties of an anti-Thy-1 monoclonal antibody. Possible analogy to OKT3/Leu-4. J. Exp Med. 159:716.

3. Meuer, S.C., R.E. Hussey, M. Fabbi, D. Fox, O. Acuto, K.A. Fitzgerald, J.C. Hodgson, J.P. Protentis, S.F. Schlossman, and E.L. Reinherz. 1984. An alternative pathway of T cell activation: a functional role for the 50 KD T11 sheep erythrocyte receptor. Cell. 36:891.

4. Pantaleo, G., D. Olive, A. Poggi, W.J. Kozumbo, L. Moretta, and A. Moretta. 1987. Transmembrane signalling via the Ti1 dependent pathway of human T cell activation: evidence for the involvement of 1,2, diacylglycerol and inositol phosphates. Eur. J. Immunol. 17:55.

5. Gardner, P.A., A. Alcover, C.M. Weyland, J. Goronzt, and E.L. Reinherz. 1988. Triggering of T lymphocytes via either T3-Ti or Ti1 surface structures opens a voltage-insensitive plasma membrane calcium-permeable channel: requirement for IL-2 gene function. J. Biol. Chem. 264:1068.

6. Ley, S.C., A.A. Davies, B. Druker, and M.J. Crumpton. 1991. The T cell receptor/CD3 complex and CD2 stimulate the tyro-
sine phosphorylation of indistinguishable patterns of polypeptides in the human T leukemic cell line Jurkat. Eur J. Immunol. 21:2203.

7. Breitmeyer, J.B., J.F. Daley, H.B. Levine, and S.F. Schlossman. 1987. The T11 (CD2) molecule is functionally linked to the T3/Ti T cell receptor in the majority of T cells. J. Immunol. 139:2899.

8. Alcover, A., C. Alberini, O. Acuto, L.K. Clayton, C. Transy, G. Spagnoli, P. Moingeon, P. Lopez, and E.L. Reinherz. 1988. Interdependence of T3-Ti and T11 activation pathways in human T lymphocytes. EMBO (Eur. Mol. Biol. Organ.) J. 7:1973.

9. Bockenstedt, L.K., M.A. Goldsmith, M. Dustin, D. Olive, T.A. Springer, and A. Weiss. 1988. The CD2 ligand LFA-3 activates T cells but depends on the expression and function of the antigen receptor. J. Immunol. 141:1904.

10. Moingeon, P., H.C. Chang, P.H. Sayre, L.K. Clayton, A. Alcover, P. Gardner, and E.L. Reinherz. 1989. The structural biology of CD2. Immunol. Rev. 111:111.

11. Frank, S.J., B.B. Niklinska, D.G. Orloff, M. Mercep, J.D. Ashwell, and R.D. Klausner. 1990. Structural mutations of the T cell receptor θ chain and its role in T cell activation. Science (Wash. DC). 249:174.

12. Bauer, A., D.J. McConkey, F.D. Howard, L.K. Clayton, D. Novick, S. Koyasu, and E.L. Reinherz. 1991. Differential signal transduction via T cell receptor CD3ξ, CD3η and CD3ψ isoforms. Proc. Natl. Acad. Sci. USA. 88:3842.

13. Irving, B.A., and A. Weiss. 1991. The cytoplasmic domain of the T cell receptor θ chain is sufficient to couple to receptor-associated signal transduction pathways. Cell. 64:891.

14. Romeo, C., and B. Seed. 1991. Cellular Immunity to HIV Activated by CD4 fused to T cell or Fc receptor polypeptides. Cell. 64:3037.

15. Letourneur, F., and R.D. Klausner. 1991. T-cell and basophil activation through the cytoplasmic tail of the T-cell receptor θ family proteins. Proc. Natl. Acad. Sci. USA. 88:8905.

16. Moingeon, P., J.L. Lucich, D.J. McConkey, F. Letourneur, B. Malissen, J. Kochan, H.-C. Chang, H.-R. Rodewald, and E.L. Reinherz. 1992. CD3ξ dependence of the CD2 pathway of activation in T lymphocytes and NK cells. Proc. Natl. Acad. Sci. USA. 89:1492.

17. Sukhatme, V.P., K.C. Sizer, A.C. Vollmer, T. Hunakpiller, and J.R. Parnes. 1985. The T cell differentiation antigen Leu-2/T8 is homologous to immunoglobulin and T cell receptor variable regions. Cell. 40:591.

18. Ohashi, P., T.W. Mak, P. van den Elsen, Y. Yanagi, Y. Hoshikai, A.F. Calman, C. Terhorst, J.D. Stobo, and A. Weiss. 1985. Reconstitution of an active surface T3/T-cell antigen receptor by DNA transfer. Nature (Lond.). 316:606.

19. Weissman, A.M., D. Hou, D.G. Orloff, W.S. Modi, H. Seuanez, S.J. O'Brien, and R.D. Klausner. 1988. Molecular cloning and chromosomal location of the human T cell receptor θ chain: Distinction from the molecular CD3 complex. Proc. Natl. Acad. Sci. USA. 85:9709.

20. Clayton, L.K., L. D'Adamo, F.D. Howard, M. Sieh, R.E. Hussey, S. Koyasu, and E.L. Reinherz. 1991. CD3η and CD3ξ are alternatively spliced products of a common genetic locus and are transcriptionally and/or post-transcriptionally regulated during T-cell development. Proc. Natl. Acad. Sci. USA. 88:5202.

21. Grynkievič, G., M. Poenie, and R.Y. Tsien. 1985. A new generation of Ca²⁺ indicators with greatly improved fluorescent properties. J. Biol. Chem. 260:3440.

22. Marrack, P., R. Endres, R. Shimonkevitz, A. Zlotnik, D. Alyanas, F. Fitch, and J. Kappler. 1985. The major histocompatibility complex-restricted antigen receptor on T cells: role of the L3T4 product. J. Exp. Med. 158:1077.

23. Siliciano R.F., J.C. Pratt, R.E. Schmidt, J. Ritz, and E.L. Reinherz. 1985. Activation of cytolytic T lymphocyte and natural killer cell function through the T11 sheep erythrocyte binding protein. Nature (Lond.). 317:428.

24. Lanier, L.L., G. Yu, and J.H. Phillips. Co-association of CD3ξ with a receptor (CD16) for IgG Fc on human natural killer cells. 1989. Nature (Lond.). 342:803.

25. Arulanandam, A.R.N., S. Koyasu, and E.L. Reinherz. T cell receptor-independent CD2 signal transduction in FcR⁺ cells. 1991. J. Exp. Med. 173:859.

26. Spruyt, L.L., M.J. Glennie, A.D. Beyers, and A.F. Williams. Signal transduction by the CD2 antigen in T cells and natural killer cells: requirement for expression of a functional T cell receptor or binding of antibody Fc to the Fc receptor, FcyRIIIA (CD16). 1991. J. Exp. Med. 174:1407.

27. Chang, H.C., P. Moingeon, P. Lopez, H. Krasnow, C. Stebbins, and E.L. Reinherz. 1989. Dissection of the human CD2 intracellular domain: identification of a segment required for signal transduction and IL-2 production. J. Exp. Med. 168:2077.