hydrolysis is not essential for at least some T cell functions, and that alternative second messenger systems that are coupled to the TCR-CD3 complex are likely.

REFERENCES AND NOTES

1. H. Festenstein, *Transplant Proc* **8**, 339 (1976).
2. K. Molnar-Kimber and J. Sprent, *J Exp Med* **151**, 407 (1980).
3. C. A. Janeway, E. A. Lerner, J. M. Jason, B. Jones, *Immunogenetics* **10**, 481 (1980).
4. J. W. Kappler, U. Staerz, J. White, P. C. Marrack, *Nature* **332**, 35 (1988).
5. H. R. MacDonald *et al.*, *ibid* , p. 40.
6. J. Imboden, C. Weyand, J. Goronzy, *J Immunol* **138**, 1322 (1987).
7. W. J. Kozumbo, D. J. Harris, S. Gromkowski, J-C. Cerottini, P. A. Cerutti, *ibid.*, p. 606.
8. S. Treves *et al* , *J. Exp Med* **166**, 33 (1987).
9. A. M. O'Rourke and M. F. Mescher, *J. Biol. Chem.* **263**, 18594 (1988).
10. J. J. Sussman *et al* , *Nature* **334**, 625 (1988).
11. $V_\beta6^+$ CBA/Ca T cells were purified by panning lymph node T cells [MAb J11d (*23*), 10-2.16 (Mab to Ia) (*24*), and complement-treated] on plastic plates coated with the MAb RR47 to $V_\beta6$ (*25*). These cells were cultured (*14*) for 14 days with irradiated (3000 rad) CBA/J (*H-2*^{k}, *Mls*$^{a/c}$) spleen cells before use in the assays. Viable cells were isolated on Ficoll-Hypaque density gradients and assayed for proliferation or [^{3}H]inositol phosphate production as indicated.
12. T cells were labeled with [^{3}H]myo-inositol by resuspending at 20×10^6 per milliliter in inositol-free Eagles basal medium (M.A. Bioproducts) containing 10% (v/v) dialyzed fetal bovine serum (Hyclone), supplements (*9*), and 5 μCi per 10^6 cells [^{3}H]myo-inositol (S.A. 60-100 Ci/mmol, Amersham), and incubating at 37°C for 3 hours. Cells were then washed extensively and incubated in medium containing 10 mM LiCl for 15 min before addition of spleen stimulators. Incubations were stopped by addition of 0.1 ml of 0.22 M HCl and 1.5 ml of chloroform:methanol (1:2 v/v). [^{3}H]Inositol phosphates in the aqueous fraction were separated by Dowex (formate) ion–exchange chromatography (*9*).
13. S. R. Webb and J. Sprent, *J. Exp. Med.* **165**, 584 (1987).
14. S. R. Webb, A. Okamoto, Y. Ron, J. Sprent, *ibid.* **169**, 1 (1989).
15. D. E. Speiser, R. Schneider, H. Hengartner, H. R. MacDonald, R. M. Zinkernagel, *ibid.* **170**, 595 (1989).
16. S. R. Webb *et al.*, *ibid* **154**, 1970 (1981).
17. M. E. Katz and C. A. Janeway, *J. Immunol.* **134**, 2064 (1985).
18. S. R. Webb, A. Okamoto, J. Sprent, *ibid* **141**, 1828 (1988).
19. C. A. Janeway *et al.*, *Immunol. Rev.* **107**, 61 (1989).
20. S. R. Webb, unpublished observations.
21. M. Baniyash, P. Garcia-Morales, J. S. Bonifacino, L. E. Samelson, R. D. Klausner, *J. Biol. Chem.* **263**, 9874 (1988).
22. M. Mercep *et al* , *Science* **242**, 571 (1988).
23. J. Bruce, F. W. Symington, T. J. McKearn, J. Sprent, *J Immunol* **127**, 2496 (1981).
24. V. T. Oi, P. P. Jones, J. W. Goding, L. A. Herzenberg, L. A. Herzenberg, *Curr. Top. Microbiol. Immunol* **81**, 115 (1978).
25. O. Kanagawa, E. Palmer, J. Bill, *Cell. Immunol.* **119**, 412 (1989).

26. This work was supported by grants from the National Institutes of Health, National Institute of Allergy and Infectious Diseases to M.F.M.

13 February 1990; accepted 9 May 1990

Structural Mutations of the T Cell Receptor ζ Chain and Its Role in T Cell Activation

STUART J. FRANK, BARBARA B. NIKLINSKA, DAVID G. ORLOFF, MLADEN MERĆEP, JONATHAN D. ASHWELL, RICHARD D. KLAUSNER

T cell hybridomas that express ζζ, but not ζη, dimers in their T cell receptors (TCRs) produce interleukin-2 (IL-2) and undergo an inhibition of spontaneous growth when activated by antigen, antibodies to the receptor, or antibodies to Thy-1. Hybridomas without ζ and η were reconstituted with mutated ζ chains. Cytoplasmic truncations of up to 40% of the ζ molecule reconstituted normal surface assembly of TCRs, but antigen-induced IL-2 secretion and growth inhibition were lost. In contrast, cross-linking antibodies to the TCR activated these cells. A point mutation conferred the same signaling phenotype as did the truncations and caused defective antigen-induced tyrosine kinase activation. Thus, ζ allows the binding of antigen/major histocompatibility complex (MHC) to αβ to effect TCR signaling.

THE TCR IS A MULTIMERIC COMplex that recognizes peptide antigens bound to MHC-encoded proteins on the surface of antigen-presenting cells. Three types of polypeptide chain make up the subunits of this receptor. The disulfide-linked αβ heterodimer forms the clonotypic antigen recognition unit, while the invariant chains of CD3, consisting of γ, δ, ε, and ζ and η, are presumably responsible for coupling ligand binding to signaling pathways that result in T cell activation and the elaboration of the cellular immune response. The ζ chain is a 16-kD nonglycosylated protein that exists in two forms in the receptor complex (*1, 2*). Eighty to 90% of receptors contain ζ homodimers, while the remainder contain ζ disulfide linked to the 22-kD η chain, a distinct protein that is related to ζ (*3*). The ζ chain is structurally unrelated to the three homologous CD3 chains. The distinctness of ζ is further emphasized by the finding that it is expressed in the absence of CD3 on NK cells (*4*). There it appears to be part of a complex with CD16, a receptor for immunoglobulin G (IgG) (*5*), that is capable of transducing signals. These observations suggest that ζ may represent an even more widely used coupling molecule than formerly thought.

Variants of the antigen-specific murine T cell hybridoma 2B4.11 have been isolated that fail to synthesize any detectable ζ or η chains and express only 2 to 5% of surface TCR found on the parental cells (*6*). When the full-length ζ cDNA is transfected into these cells, a structurally normal TCR is transported to the surface (*7*) and antigen induces interleukin-2 (IL-2) production and a G_1/S cell cycle block (growth inhibition). Antigen does not induce apoptosis in these η-deficient cells (*8*), and, as previously reported, the lack of η correlated with the absence of antigen-induced phosphoinositide hydrolysis (*9*). Because these reconstituted cells could signal to produce IL-2 in the absence of any detectable η, we were encouraged to analyze further the signaling role of ζ by reconstitution using cDNAs encoding structurally altered ζ chains. Two truncated proteins, designated CT108 and CT150, were designed by changing the codons for residues Glu^{108} and Lys^{150} to termination codons (Fig. 1A) (*10*). The products of these cDNAs and the full-length ζ (FL), were analyzed after transient transfection into COS cells by metabolic labeling and immunoprecipitation with antibodies to a ζ peptide (*11*); the transfectants expressed either full-length ζ or ζ chains with the predicted truncations (Fig. 1B).

The ζ cDNA constructs were transfected by electroporation into the ζη-deficient variant of 2B4.11, named 2M.2. The presence of the predicted ζ proteins in these transformants was verified by immunoprecipitation and immunoblotting with the antibody to ζ peptide 2 (Fig. 2A). The full-length transformant produced a 16-kD ζ chain, whereas the only ζ chains detected in CT150 and CT108 were consistent with the predicted sizes of the proteins encoded by the mutated cDNAs. In addition, the immunoblotting verified the absence of η in these transformants (Fig. 2A). The transformants were iodinated to evaluate the structural components of the assembled surface complexes. After immunoprecipitation with monoclonal antibodies (MAbs) to the α or ε chains,

S. J. Frank, D. G. Orloff, R. D. Klausner, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.
B. B. Niklinska, M. Merćep, J. D. Ashwell, Biological Response Modifiers Program, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

or with an antiserum to a ζ peptide, and separation of TCR proteins on 2D diagonal gels, we found that homodimers of ζ were on the surface of the respective transformants. In all cases the ζ chains were assembled normally into surface complexes. The role of ζ in surface expression of the TCR complex involves the sparing of the αβγδε pentameric structure from rapid lysosomal degradation (*7, 12*). When ζ is present, the heptameric complex is targeted to the plasma membrane. The TCR surface expression in CT108 shows that 40% of the cytoplasmic tail of ζ can be removed without altering this targeting function.

We investigated signal transduction of TCRs containing truncated ζ chains by assaying IL-2 production and the inhibition of spontaneous growth. Stimulation of FL, CT108, and CT150 cells with antigen revealed a hierarchy of IL-2 production (Fig. 3A). FL cells generated detectable IL-2 when stimulated with as little as 1 μM pigeon cytochrome c fragment 81–104 [PCc (81–104)]. CT150 cells produced little IL-2, and only at relatively high antigen concentrations. CT108 cells consistently produced no IL-2, even with antigen concentrations of 30 μM. All three cells were responsive to the pharmacologic inducers of IL-2, phorbol esters plus calcium ionophore (*13*). Stimulation with an immobilized MAb to CD3, 145-2C11 (2C11), gave different results (Fig. 3B). FL cells still produced the most IL-2, with plateau levels within twofold of that induced by antigen. In contrast to antigen, 2C11 induced both CT150 and CT108 cells to produce easily detectable IL-2, although less than FL cells (maximal units produced by CT150 and CT108 cell were 15 to 20% of that produced by FL). Whereas CT108 cells never produced IL-2 when stimulated with antigen, they were at least as good, if not better, than CT150 when responding to MAb against CD3. The dose response to 2C11 was essentially identical for all three cells. The different pattern of response to antigen and 2C11 could be because the former binds αβ and the latter binds CD3-ε. Alternatively, cross-linking with antibodies may be qualitatively different from occupying the TCR with ligand. To test this, an immobilized MAb to the α subunit A2B4-2 was used as a stimulant; this reagent stimulated similar amounts of IL-2 production from all of the cell lines. H57-597 (*14*) (H57), a MAb to αβ, stimulated a pattern of IL-2 production indistinguishable from A2B4-2 (*13*). G7, a mitogenic MAb to Thy-1, stimulated IL-2 production from T cells, although the maximal levels it can generate from 2B4.11 are lower than those elicited by antigen or antibodies to the TCR (*6*). G7 is unusual in that it does not require external cross-linking to activate the cells (*15*). In contrast to the antibodies to the TCR, G7 caused only the FL cells to produce IL-2 (Fig. 3D). In addition, we tested the response of the FL and CT108 cells to the "superantigen" staphylococcal enterotoxin B, which reacts with T cell receptors on the basis of V_β rather than antigen specificity (*16*). FL cells produced readily detectable amounts of IL-2 in response to this stimulus, whereas CT108 produced no IL-2 at any concentration up to 1 μg of the enterotoxin per milliliter (*13*). The inhibition of growth after TCR stimulation is another manifestation of T cell activation in these transformed cells (*17*). Antigen caused significant growth inhibition in FL cells (Fig. 3E). CT150 cells were slightly inhibited, and only at high concentrations of antigen, whereas CT108 cells were not inhibited at all. In contrast to the results with antigen, stimulation with immobilized antibodies to T cell receptor (Fig. 3, F and G) caused each of the cells to slow their growth. The effects of the antibodies on the different transformants were identical in magnitude and concentration dependence.

The effect of ζ truncation on the ability of ligand to induce IL-2 secretion or growth inhibition is most likely a consequence of inadequate or absent signal transduction. These cells, which lack η, show no phosphatidylinositol (PI) breakdown in response to antigen plus MHC (*13*). However, η is not required for the activation of the T cell receptor–stimulated protein tyrosine kinase pathway (*9*), as monitored by the tyrosine phosphorylation of ζ itself (*18*). To assess this, we made point mutations of the region of ζ implicated as functional by the truncation mutants, particularly in the region that contains a possible consensus binding sequence for nucleotides (Gly-X-Gly-X-X-Gly . . . Ala-X-Lys) found between amino acids 135 and 150 (*19*). We have replaced Lys^{150} with Arg with no effect on T cell receptor function (*13*). Lys^{150} would be analogous to the critical amino acid for phosphotransferase activity found in most kinases (*20*).

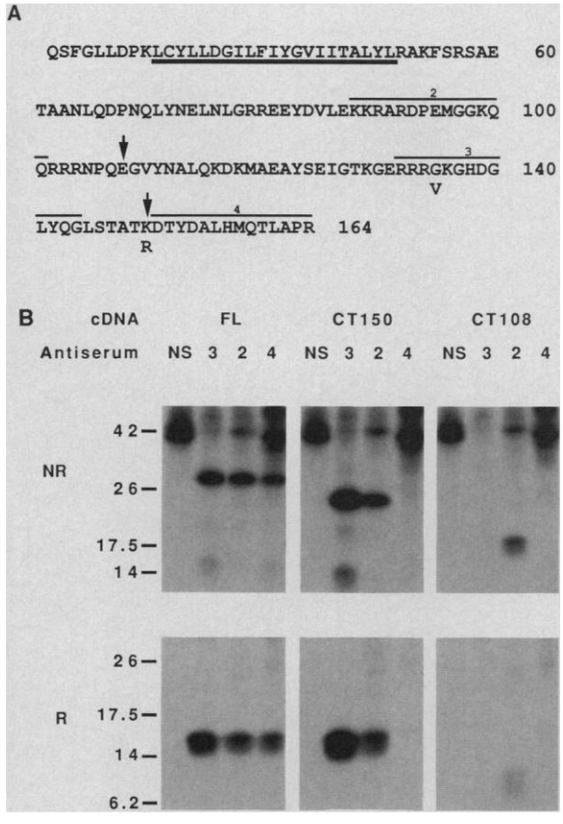

Fig. 1. (**A**) Map of ζ mutations. The deduced amino acid sequence of the mature native murine ζ chain is shown (the 21-residue leader sequence is omitted) (*19*). Truncations at residues 108 (CT108) and 150 (CT150) with termination codons replacing the native Glu (E) and Lys (K), respectively, are indicated by arrows. Point mutations (GV135 and KR150) changing residues Gly^{135} to Val^{135} and Lys^{150} to Arg^{150} are indicated by placement of the abbreviation for the mutated residue below that for the native residue. Peptides 2, 3, and 4, to which antisera were made, are indicated by solid lines above the included residues. The transmembrane region is underlined. M13-based, oligonucleotide-directed site-specific mutagenesis was done with the Bio-Rad Mutagene Kit and the resulting mutant ζ cDNAs were selected by dideoxy sequencing. These mutant cDNAs (or the full-length native ζ cDNA) were cloned into the pSVL (Pharmacia) and pfNEO (*21*) (FL, CT108, and CT150) or RSV.5NEO (GV135 and KR150) expression vectors and the entire protein-coding regions of these constructs were resequenced to verify the presence of only the intended mutations before transfection. (**B**) Expression of truncated ζ chains in COS-1 cells. The cDNAs (10 μg) encoding FL, CT108, or CT150 in the pSVL expression vector were transfected by the calcium phosphate precipitation method (*22*) into COS-1 cells (one 150-mm^2 dish per transfection). The cells were treated with 5 mM sodium butyrate for 18 hours beginning 24 hours after transfection and were then metabolically labeled with [^{35}S]methionine for 45 min. After solubilization in 0.5% (w/v) Triton X-100, 0.3 M NaCl, 50 mM tris-HCl, pH 7.4, and protease inhibitors, equal volumes of this labeled lysate were immunoprecipitated with antibodies to ζ peptides 2 (directed at residues 88–101 of ζ), 3 (residues 132–144), and 4 (residues 151–164), and a normal rabbit serum (NS). Eluates were divided and separated either nonreduced (NR) or reduced (R) on 15% acrylaide gels and exposed by autoradiography.

However, when Gly[135] was replaced with Val (GV135), the activation phenotype was similar to that of the truncations (Fig. 4A). Treatment of this cell with antigen or antibody to the Thy-1 resulted in minimal IL-2 output (up to 1 unit), while antibodies to TCR elicited appreciable IL-2. FL cells again responded to antigen, antibody to Thy-1, and antibody to the T cell receptor cross-linking with robust IL-2 output (*13*). The altered ζ cDNA in GV135 still encodes a full-length protein containing all of the Tyr residues. Both FL and GV135 are Tyr-phosphorylated in response to 2C11, with the expected molecular size shift from 16 to 21 kD (Fig. 4B). Thus, the GV135 mutant ζ chain is a substrate for the tyrosine kinase. In contrast, only the wild-type ζ is Tyr-phosphorylated in response to antigen. Whereas antigen is a less potent stimulus of ζ phosphorylation than 2C11, even overexposure of the autoradiograms failed to reveal any antigen-induced ζ phosphorylation in GV135. Although it is still unknown whether ζ can bind nucleotides, these results show that this region of the cytoplasmic tail is important to allow ζ to transduce the occupancy of the receptor to cell activation.

Could the difference in response to antigen and antibodies to the T cell receptor between FL and the truncated ζ mutants reflect merely a difference in the potency of the stimuli? This explanation can be rejected on several grounds. Although the maximal FL IL-2 output to 2C11 was approximately twofold greater than that induced by antigen (~200 units compared to ~100 units), its maximal response to A2B4-2 was indistinguishable from antigen, indicating a lack of fundamental difference in the potency of the stimuli. Dose-response profiles for each of the stimuli indicated that the full-length and mutant receptor (CT108, for example) transduced both the 2C11- and A2B4-2–induced activating signals equally well. The antigen-induced IL-2 dose-response profiles for FL and CT108, however, were fundamentally different with at least a 100-fold (and perhaps infinite) shift in CT108 responsiveness (no detectable IL-2 output at the highest antigen concentration). The growth-inhibition assay exhibited the same dichotomy with CT108 and FL, displaying identical dose-response profiles for antibodies to the T cell receptor but CT108 lacking growth inhibition at any antigen concentration.

These results lead us to propose a two-state model for the activation of the T cell receptor by physiologic ligand. Binding of the T cell receptor to antigen/MHC structurally alters the receptor in a manner that does not lead to activation without the concerted function of the cytoplasmic tail of ζ. Whatever function ζ performs appears to be bypassed by direct external cross-linking of the T cell receptor by antibodies to the T cell receptor. We hypothesize that, in the presence of an intact ζ chain, the activated state of the T cell receptor may be identical regardless of the stimulus. According to this model, the role of ζ is not to couple to second messenger–generating systems, but rather to complete the ligand-induced structural changes in the receptor, perhaps by mediating internal cross-linking of the receptor to cytoskeletal elements. Such a model does not claim that ligand does not induce

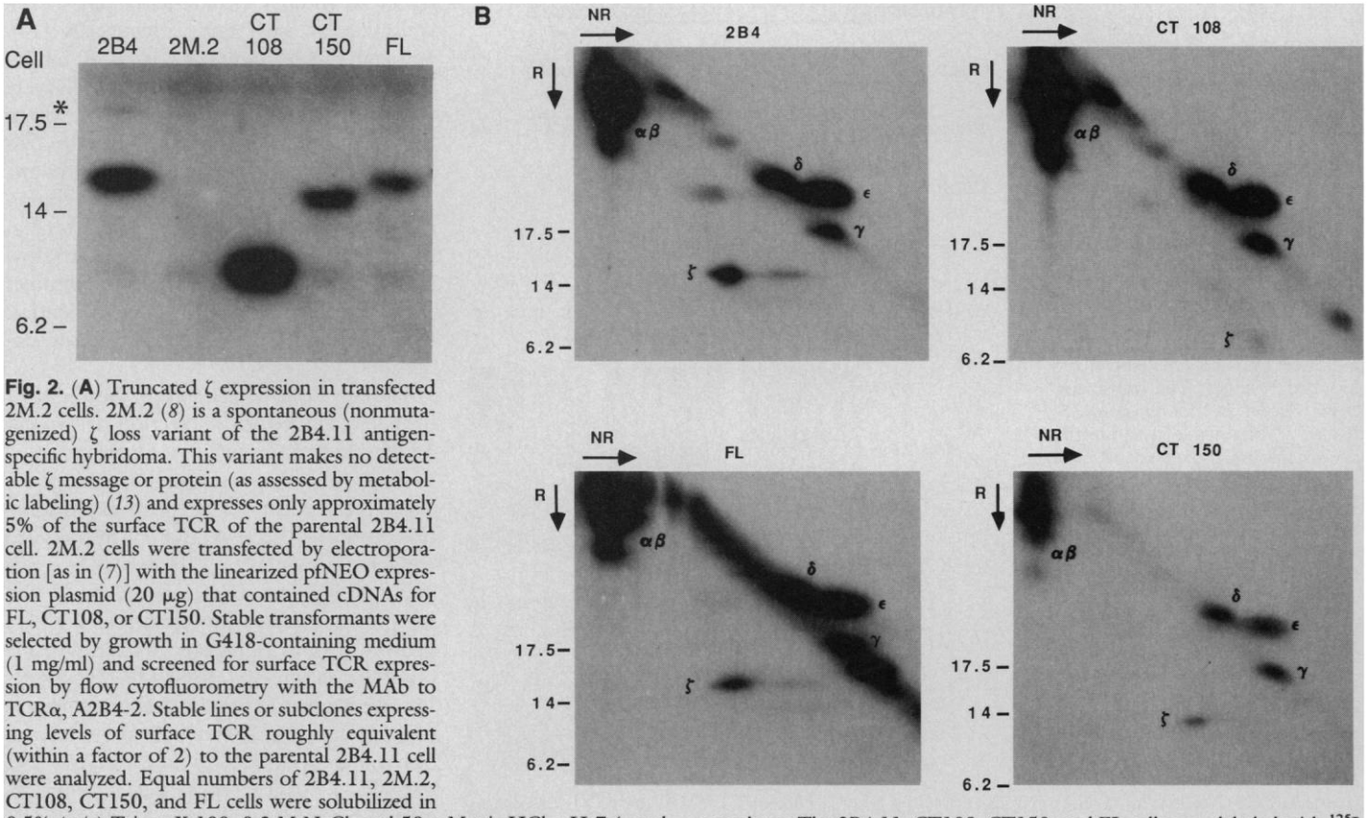

Fig. 2. (**A**) Truncated ζ expression in transfected 2M.2 cells. 2M.2 (*8*) is a spontaneous (nonmutagenized) ζ loss variant of the 2B4.11 antigen-specific hybridoma. This variant makes no detectable ζ message or protein (as assessed by metabolic labeling) (*13*) and expresses only approximately 5% of the surface TCR of the parental 2B4.11 cell. 2M.2 cells were transfected by electroporation [as in (*7*)] with the linearized pfNEO expression plasmid (20 μg) that contained cDNAs for FL, CT108, or CT150. Stable transformants were selected by growth in G418-containing medium (1 mg/ml) and screened for surface TCR expression by flow cytofluorometry with the MAb to TCRα, A2B4-2. Stable lines or subclones expressing levels of surface TCR roughly equivalent (within a factor of 2) to the parental 2B4.11 cell were analyzed. Equal numbers of 2B4.11, 2M.2, CT108, CT150, and FL cells were solubilized in 0.5% (w/v) Triton X-100, 0.3 M NaCl, and 50 mM tris-HCl, pH 7.4, and centrifuged. The ζ proteins in the supernatant were immunoprecipitated with antiserum to ζ peptide 2, resolved on a 15% reducing SDS-polyacrylamide gel, transferred to nitrocellulose, and immunoblotted with the same antiserum and ^{125}I-labeled protein A. As this serum reacts with both ζ and η (*11*), the position of η in 2B4.11 cells is indicated by an asterisk. Note the absence of ζ in 2M.2; we estimate that we could readily detect 0.1% of the parental level of ζ. (**B**) Truncated ζ chains assemble into surface TCR complexes. The 2B4.11, CT108, CT150, and FL cells were labeled with ^{125}I by the lactoperoxidase–glucose oxidase method under conditions that favor intact cell labeling (*1*). After solubilization, TCR complexes were immunoprecipitated with the MAb to CD3, 2C11, and resolved by 2D nonreducing (NR)-reducing (R) SDS–polyacrylamide gel electrophoresis. The positions of the TCR and CD3 chains as well as full-length and truncated ζ chains are indicated. Precipitations with antiserum to ζ peptide 2 also showed all the indicated chains.

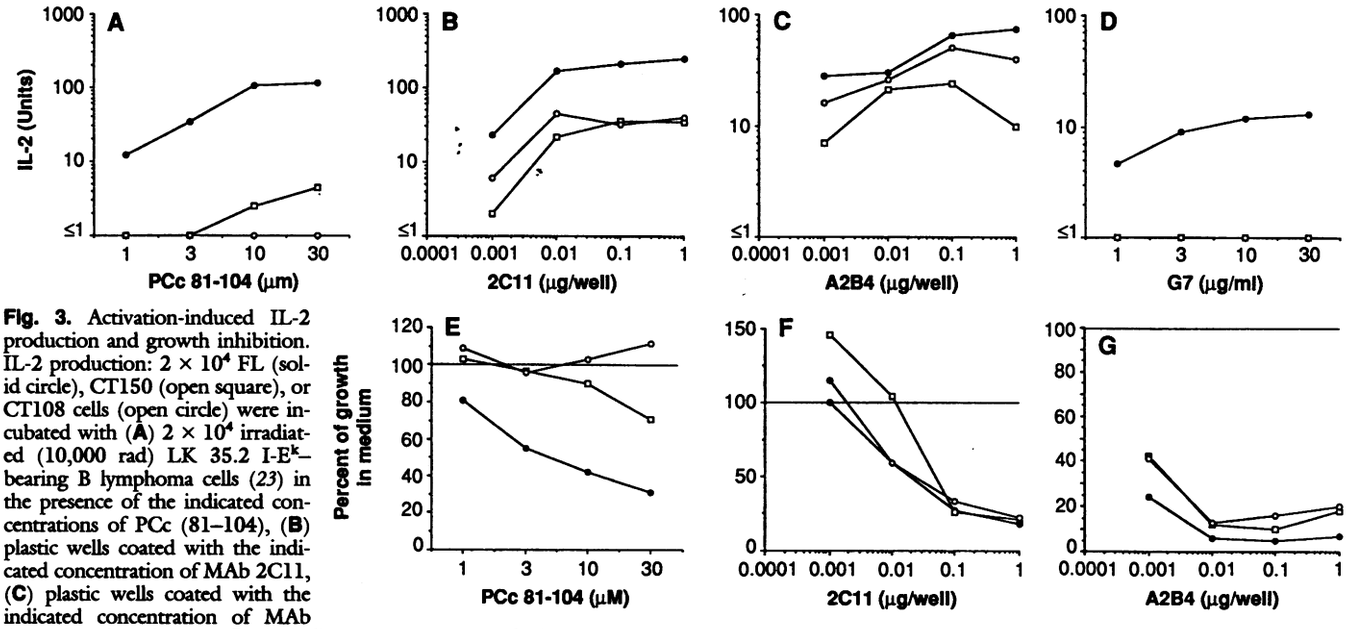

Fig. 3. Activation-induced IL-2 production and growth inhibition. IL-2 production: 2×10^4 FL (solid circle), CT150 (open square), or CT108 cells (open circle) were incubated with (**A**) 2×10^4 irradiated (10,000 rad) LK 35.2 I-E^k–bearing B lymphoma cells (*23*) in the presence of the indicated concentrations of PCc (81–104), (**B**) plastic wells coated with the indicated concentration of MAb 2C11, (**C**) plastic wells coated with the indicated concentration of MAb A2B4-2 to the TCRα, or (**D**) the indicated concentration of soluble MAb G7 to Thy-1. After 24 hours, samples of supernatant were removed and IL-2 content was determined (*17*). Growth inhibition: After the removal of supernatant for IL-2 assay, the T hybridoma cells activated with (**E**) antigen, (**F**) immobilized 2C11, or (**G**) immobilized A2B4-2 were assayed for their ability to incorporate [^{3}H]thymidine (*17*). For each assay (A to G), at least three separate experiments yielded similar results. Fluorescence-activated cell sorter analysis of FL and CT108 cells showed that both have the following phenotype: $CD4^-$, $CD8^-$, $CD2^-$, LFA 1^+, Thy-1^+, $CD45^+$, TCR^+. The intensity of staining for each of the latter four markers was comparable for both cell types.

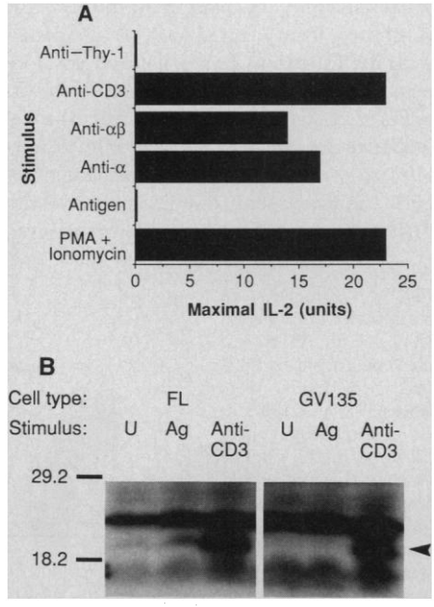

Fig. 4. (**A**) GV135 cells (2×10^4) were incubated with phorbol myristate acetate (PMA) (10 ng/ml) and various amounts of ionomycin, or various amounts of MAbs to Thy-1 (G7), TCRαβ (H57), TCRα (A2B4-2), CD3-ε (2C11), or antigen [2×10^4 irradiated LK 35.2 cells plus PCc (81–104)]. IL-2 production was measured as in Fig. 3. A full concentration-dependence curve was done for each type of stimulus; for simplicity, only the maximal response in each group is shown. (**B**) Tyrosine kinase activation in GV135. Equal numbers of FL or GV135 cells were incubated at 37°C with LK 35.2 B cells (T:B ratio = 2:1) and were unstimulated (U) or stimulated with PCc (81–104) (Ag) (100 μM) or a 1:25 dilution of MAb to CD3-ε, 2C11 (anti-CD3), supernatant for 30 min. Cells were then washed twice with ice-cold phosphate-buffered saline, pH 7.4, in the presence of 0.4 mM sodium orthovanadate plus 0.4 mM EDTA. They were then solubilized as in Fig. 2A in the presence of 1 mM vanadate and 2 mM EDTA at 1×10^8 T cells per milliliter. Samples were then mixed with Laemmli sample buffer and the cell lysate of 5×10^6 cell equivalents per sample was resolved on 15% SDS-polyacrylamide gels, transferred to nitrocellulose, and immunoblotted with an antiserum to phosphotyrosine (*24*) and ^{125}I-labeled protein A. Autoradiograms were exposed for 2 days (FL samples) or 3 days (GV135 samples). Phospho ζ (arrowhead) appears at approximately 21 kD (*18*) in the 2C11 stimulation of both cell types and in the antigen stimulation of FL only.

receptor cross-linking, but rather that, in the absence of ζ, this effect is not sufficient for activation. The apparent qualitative differences between antibodies to the T cell receptor and ligand must raise a cautionary note in the interpretation of T cell activation studies that use only antibodies to the T cell receptor.

REFERENCES AND NOTES

1. L. E. Samelson, J. B. Harford, R. D. Klausner, *Cell* **43**, 223 (1985).
2. H. C. Oettgen, C. L. Pettey, W. L. Meloy, C. Terhorst, *Nature* **320**, 272 (1986).
3. M. Baniyash, P. Garcia-Morales, J. S. Bonifacino, L. E. Samelson, R. D. Klausner, *J. Biol. Chem.* **263**, 9874 (1988).
4. P. Anderson, M. Caligiuri, J. Ritz, S. K. Schlossman, *Nature* **341**, 159 (1989).
5. L. L. Lanier, G. Yu, J. H. Phillips, *ibid.* **342**, 803 (1989).
6. J. J. Sussman *et al.*, *Cell* **52**, 85 (1988).
7. A. M. Weissman *et al.*, *EMBO J.* **8**, 3651 (1989).
8. M. Merćep, A. M. Weissman, S. J. Frank, R. D. Klausner, J. D. Ashwell, *Science* **246**, 1162 (1989).
9. M. Merćep *et al.*, *ibid.* **242**, 571 (1988).
10. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
11. D. G. Orloff, S. J. Frank, F. A. Robey, A. M. Weissman, R. D. Klausner, *J. Biol. Chem.* **264**, 14812 (1989).
12. Y. Minami, A. M. Weissman, L. E. Samelson, R. D. Klausner, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2688 (1987); J. Sussman *et al.*, *Cell* **52**, 85 (1988).
13. S. J. Frank, unpublished data.
14. R. T. Kubo, W. Born, J. W. Kappler, P. Marrack, M. Pigeon, *J. Immunol.* **142**, 2736 (1989).
15. K. C. Gunter, T. R. Malek, E. M. Shevach, *J. Exp. Med.* **159**, 716 (1984).
16. J. White *et al.*, *Cell* **56**, 27 (1989).
17. J. D. Ashwell, R. E. Cunningham, P. D. Noguchi, D. Hernandez, *J. Exp. Med.* **165**, 173 (1987).
18. L. E. Samelson, M. D. Patel, A. M. Weissman, J. B. Harford, R. D. Klausner, *Cell* **46**, 1083 (1986); M. Baniyash, P. Garcia-Morales, E. Luong, L. E. Samelson, R. D. Klausner, *J. Biol. Chem.* **263**, 18225 (1988).
19. A. M. Weissman *et al.*, *Science* **239**, 1018 (1988).
20. S. K. Hanks, A. M. Quinn, T. Hunter, *ibid.* **241**, 42 (1988).
21. T. Saito, A. Weiss, J. Miller, M. A. Norcross, R. N. Germain, *Nature* **325**, 125 (1987).
22. C. M. Gorman, L. F. Moffat, B. H. Howard, *Mol. Cell. Biol.* **2**, 1044 (1982).
23. J. Kappler, J. White, D. Wegmann, E. Mustain, P. Marrack, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3604 (1982).
24. E. D. Hsi *et al.*, *J. Biol. Chem.* **264**, 10836 (1989).

25. We thank E. Long for RSV.5NEO. We also thank L. Samelson and J. Bonifacino for helpful discussions and E. Perry and T. Koomson for expert assistance in preparation of this manuscript.

11 January 1990; accepted 2 May 1990