deed involved in GTP hydrolysis (*17*). Rap1A-p21 (T61Q), unlike wild-type Rap1A-p21, is slightly activated by GAP (Fig. 4), although the GAP-activated GTP hydrolysis is still much slower than that of Ras-p21 (Fig. 1). In addition, the affinity of Rap1A-p21 (T61Q) for GAP was greatly decreased (K_i = 1 ± 0.20 μm).

In summary, we have found that the protein product of the K*rev*-1 gene, Rap1A-p21, acts as a potent inhibitor of GAP-mediated activation of Ras-p21 GTPase activity. In platelets (*18*) the concentration of Rap1A-p21 is at least ten times as high as that of Ras-p21, suggesting that, in vivo, rap1A-p21 might limit the amount of GAP available for interaction with Ras-p21. Because GAP may be necessary for *ras* action, K*rev*-1 may thus suppress transformation of cells by *ras* oncogenes. Conversely, the amount of GAP available for binding to Rap1A-p21 may be limited in cells expressing high concentrations of oncogenic Ras proteins that have high affinities for GAP (*5*).

Interaction between GAP and Rap1A-p21 was shown here to be GTP-dependent, as is the interaction of GAP with Ras-p21. However, association of Rap1A-p21 with GTP is likely to be controlled, in part, by its own GTPase activating protein (smg-p21-GAP, Rap-GAP) (*19*). Biological activity of Ras-p21 may therefore be modulated directly by competition for GAP and indirectly by the effect of Rap-GAP on Rap1A-p21.

We propose the following model for GAP function. First, GAP binding to Ras-p21.GTP may cause a conformational change in Ras that is an intermediate in the GTP hydrolysis reaction (*20*). This change likely involves amino acids around position 61 of ras-p21, because this region appears to be involved in GTP hydrolysis (*17*). According to basic thermodynamic considerations, GAP itself is expected to undergo a conformational change upon binding. This form of GAP ("GAP*") then perhaps serves as the effector of Ras-p21 action. GAP* may also interact with other effector molecules, or would itself generate second-messenger molecules. Hydrolysis of GTP by Ras-p21 terminates the GAP* state. When GAP binds to Rap1A-p21.GTP, these conformational changes do not occur, the GAP* state is not achieved, and activation of GTP hydrolysis cannot take place.

REFERENCES AND NOTES

1. M. Trahey and F. McCormick, *Science* **238**, 542 (1987).
2. J. B. Gibbs *et al.*, *Proc Natl Acad Sci U S A* **85**, 5026 (1988).
3. H. Adari, D. R. Lowy, B. M. Willumsen, C. J. Der, F. McCormick, *Science* **240**, 518 (1988).
4. C. Cales, J. F. Hancock, C. J. Marshall, A. Hall, *Nature* **332**, 548 (1988).
5. U. Vogel *et al.*, *ibid* **335**, 90 (1988).
6. J. B. Gibbs *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6630 (1989).
7. H. Kitayama, Y. Sugimoto, T. Matsuzaki, Y. Ikawa, M. Noda, *Cell* **56**, 77 (1989).
8. V. Pizon, P. Chardin, I. Lerosey, B. Olofsson, A. Tavitian, *Oncogene* **3**, 201 (1988).
9. M. Kawata *et al.*, *J Biol Chem* **264**, 9133 (1989).
10. K. R. Halliday, *J Cyclic Nucleotide and Protein Phosphorylation Res* **9**, 435 (1983); A. G. Gilman, *Annu Rev Biochem* **56**, 615 (1987); T. E. Dever, M. J. Glynias, W. C. Merrick, *Proc Natl Acad Sci U S A* **84**, 1814 (1987).
11. The GTP hydrolysis rates of the various proteins were determined at least three times.
12. J. John, I. Schlichting, E. Schiltz, P. Rosch, A. Wittinghofer, *J. Biol. Chem.* **263**, 11792 (1988).
13. For the inhibition studies the single rate contants were determined by initial rate measurements under steady-state conditions. The determinations of the rate constants have an error of 10%. The K_i value of wild-type Rap-p21 was determined as the average of seven independent experiments under various Ras-p21 and GAP concentrations.
14. E. F. Pai *et al.*, *Nature* **341**, 209 (1989); L. Tong, M. V. Milburn, A. M. De Vos, S.-H. Kim, *Science* **245**, 244 (1989).
15. G. A. Petsko and A. Wittinghofer, unpublished data.
16. A. T. Brunger, J. Kuriyan, M. Karplus, *Science* **235**, 458 (1987).
17. E. F. Pai, U. Krengel, G. A. Petsko, R. S. Goody, W. Kabsch, A. Wittinghofer, *EMBO J.*, in press.
18. T. Ohmori *et al.*, *J Biol. Chem.* **264**, 1877 (1989).
19. T. Ueda *et al.*, *Biochem. Biophys. Res. Commun.* **159**, 1411 (1989).
20. S. E. Neal, J. F. Eccleston, M. R. Webb, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3562 (1990).
21. J. Tucker *et al.*, *EMBO J* **5**, 1351 (1986).
22. M. Trahey *et al.*, *Science* **242**, 1697 (1988); V. A. Luckow and M. D. Summers, *Biotechnology* **6**, 47 (1988).

23. We thank D. Sanders and R. Goody for helpful discussions; G. Petsko for help with model-building of rap1A-p21; E. O'Rourke, D. Lowe, and K. Holmes for technical support.

26 January 1990; accepted 1 June 1990

Activation of Polyphosphoinositide Hydrolysis in T Cells by H-2 Alloantigen But Not MLS Determinants

ANNE M. O'ROURKE, MATTHEW F. MESCHER,* SUSAN R. WEBB

Murine minor lymphocyte-stimulating (Mls) determinants are cell surface antigens that stimulate strong primary T cell responses; the responding T cells display restricted T cell receptor (TCR) V_β gene usage. Interaction of T cells with mitogens or major histocompatibility complex (MHC) antigens activated the polyphosphoinositide (PI) signaling pathway, but this pathway was not triggered by Mls recognition. However, interleukin-2 (IL-2) secretion and proliferation to all three stimuli were comparable. Thus, although recognition of both allo–H-2 and Mls determinants is thought to be mediated by the TCR, these antigens appear to elicit biochemically distinct signal transduction pathways.

T CELLS RESPOND TO ALLOGENEIC MHC proteins and to foreign antigen in the context of self-MHC proteins. Products of the murine *Mls* loci stimulate primary T cell responses in an MHC class II–dependent, but unrestricted manner (*1–3*). The physical structure of Mls determinants is unknown; their presence is detectable only by virtue of T cell proliferative and secretory responses. T cell recognition of Mls^a determinants involves limited TCR V_β gene usage; for example, T cells bearing $V_\beta6$ and $V_\beta8.1$. TCR are selectively deleted in Mls^a mouse strains (*4, 5*). These and other data indicate that recognition of both Mls determinants and allo-MHC antigens involve TCR α/β heterodimers. The interaction of T cells with allogeneic MHC proteins (murine H-2) or foreign antigen and self H-2 molecules initiates the hydrolysis of T cell membrane PI with concomitant formation of the second messengers inositol phosphate and diacylglycerol (*6–8*). Studies with both cytotoxic and helper T lymphocytes have suggested the existence of additional, as yet undefined, TCR-coupled second messenger pathways important in generation of the functional responses to antigen (*9, 10*). By contrast, the signaling pathways coupling recognition of Mls to proliferation and secretion have not been examined.

Initial experiments that investigated transmembrane signaling by Mls^a-reactive T cells used $V_\beta6^+$ T cells purified from CBA/CA (*H-2*^{k}, *Mls*^{b}) mice, primed in vitro with Mls^a-positive CBA/J spleen cells (*H-2*^{k}, *Mls*$^{a/c}$) (*11*). These $V_\beta6^+$ T cells made secondary proliferative responses to Mls^a-bearing CBA/J and AKR/J (*H-2*^{k}, *Mls*^{a}) stimulators and to concanavalin A (Con A), but not to syngeneic CBA/Ca (*H-2*^{k}, *Mls*^{b}) cells (Fig. 1A). In parallel experiments, $V_\beta6^+$ T cells labeled with [^{3}H]myo-inositol (*12*) displayed a significant increase in [^{3}H]*PI turn-*

A. M. O'Rourke and M. F. Mescher, Division of Membrane Biology, Medical Biology Institute, La Jolla, CA 92037.
S. R. Webb, Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

*To whom correspondence should be addressed.

over, sustained for 60 min in response to concanavalin A (Con A)–pulsed cells (Fig. 1B). Mls^a-bearing AKR/J stimulators elicited only a marginal [^{3}H]inositol phosphate response by the same $V_\beta6^+$ population, and only after 30 min of incubation did this response become significantly greater than the level detected on incubation with syngeneic cells. The level of inositol phosphates detected, although low, might represent a true Mls^a-stimulated signaling mechanism. Alternatively, this low response might be directed to non-Mls^a minor antigens expressed by the stimulating strain. To address this second possibility, we examined PI hydrolysis in a T helper clone, K3.9 [B10.D2 (*H-2^d*, *Mls^b*)]. This clone can be stimulated either by Mls^a on a variety of different *H-2* haplotypes or by H-2^{p,b,q} but not H-2^{k,u,v} alloantigen (*13*). The [^{3}H]inositol phosphate response to allo–H-2 (B10.P) stimulators was rapid and sustained (Fig. 2). The inositol trisphosphate response could not be detected, since the formation of this species is rapid and it does not accumulate in the presence of LiCl. Mls-disparate stimulators (DBA/2: *H-2^d*, *Mls^a*) failed to stimulate detectable PI hydrolysis in the clone; levels of inositol phosphates were not significantly different from syngeneic stimulators (B10.D2) at any incubation time. Although we cannot rule out formation of inositol trisphosphate in response to Mls^a, its turnover is not reflected in an accumulation of the breakdown products inositol bisphosphate or inositol monophosphate (Fig. 2). The proliferative response of clone K3.9 was similar for both Mls^a and allo–H-2 stimulation (Fig. 2). These data with cloned T cells confirm the findings with purified $V_\beta6^+$ cells (Fig. 1B) and suggest that Mls^a-reactivity is not associated with significant PI turnover.

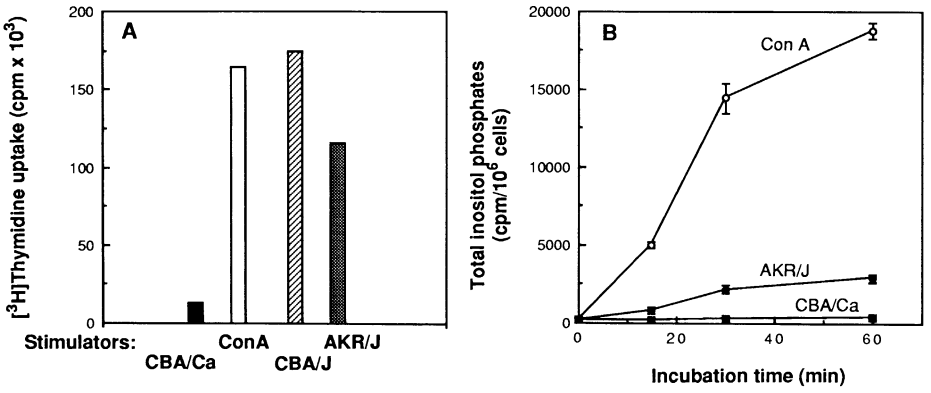

Fig. 1. (**A**) Proliferation and (**B**) [^{3}H]inositol phosphate production by $V_\beta6^+$ T cells in response to allo–class II and Mls^a determinants. (A) $V_\beta6^+$ CBA/Ca (*H-2^k*, *Mls^b*) secondary T cells (*11*) were cultured at 2.5 × 10^4 cells per well with 5 × 10^5 mitomycin C–treated spleen stimulators depleted of T cells by treatment with antibody to Thy-1 and complement as described (*14*). Con A was added at 5 μg/ml. Cultures were pulsed with 1 μCi of [^{3}H]thymidine after 48 hours and harvested 18 hours later. Results are means of triplicate cultures and SEM were less than 20% in all cases. (B) $V_\beta6^+$ T cells (10^6 per point) were labeled with [^{3}H]myoinositol (*12*) before incubation with 5 × 10^6 irradiated T-depleted spleen stimulator cells for the indicated times. [^{3}H]Inositol phosphates were extracted and purified as in Fig. 2. Stimulators were CBA/Ca (*H-2^k*, *Mls^b*) (■), AKR/J (*H-2^k*, *Mls^a*) (●), or CBA/Ca pulsed with 5 μg/ml Con A (○). Results are mean ± SEM of triplicate samples.

Table 1. IL-2 secretion and inositol phosphate production by an allo–class II and Mls^a-reactive T cell hybridoma. KW1 cells (10^6) that had been [^{3}H]inositol ([^{3}H]Ins)–labeled were incubated for the indicated times with 5 × 10^6 stimulators as described for Fig. 1. Total [^{3}H]inositol phosphates are mean ± SEM for triplicate samples. IL-2 secretion was assayed by culturing 10^5 hybridoma cells with 5 × 10^5 irradiated spleen stimulators for 24 hours. Supernatants were incubated for 24 hours with HT-2 (IL-2–dependent) cells. [^{3}H]Thymidine ([^{3}H]Tdr) was then added to the cultures, which were harvested 18 hours later (*18*). [^{3}H]Thymidine incorporation by HT-2 cells at a 1:2 dilution of hybridoma culture supernatant is shown. IL-2 units were determined by serial dilution of supernatants, with one IL-2 unit defined as the volume of supernatant required to give half-maximal proliferation.

Stimulators		Total [^{3}H]Ins		IL-2 secretion	
Strain	Haplotype	Time (min)	cpm/ 10^6 cells	[^{3}H]Tdr uptake	Unit/ ml
		Experiment 1			
		0	315 ± 67		
B10.Br	*H-2^k*, *Mls^b*	30	699 ± 152	784	<1
B10.Br	*H-2^k*, *Mls^b*	60	961 ± 192		
CBA/J	*H-2^k*, *Mls$^{a/c}$*	30	738 ± 185		
CBA/J	*H-2^k*, *Mls$^{a/c}$*	60	719 ± 79	65,942	217
B10.P	*H-2^p*, *Mls^b*	30	4262 ± 675		
B10.P	*H-2^p*, *Mls^b*	60	3751 ± 535	59,194	118
		Experiment 2			
		0	506 ± 66		
B10.Br	*H-2^k*, *Mls^b*	30	565 ± 213	856	<1
B10.Br	*H-2^k*, *Mls^b*	60	629 ± 207		
CBA/J	*H-2^k*, *Mls$^{a/c}$*	15	467 ± 254		
CBA/J	*H-2^k*, *Mls$^{a/c}$*	30	418 ± 80	59,066	143
CBA/J	*H-2^k*, *Mls$^{a/c}$*	60	385 ± 48		
B10.P	*H-2^p*, *Mls^b*	15	1252 ± 92		
B10.P	*H-2^p*, *Mls^b*	30	1843 ± 323	34,724	23
B10.P	*H-2^p*, *Mls^b*	60	1458 ± 154		

The failure to detect PI turnover in response to Mls antigens does not reflect the use of an inadequate number of stimulator cells. K3.9 showed no detectable PI hydrolysis with Mls^a-positive highly purified B cell stimulators even when responder cell:stimulator cell ratios were as high as 1:10 (Fig. 3B). With allo-H-2^p B cell stimulators, by contrast, reducing the number of stimulators by tenfold (1:1) still elicited strong PI turnover. Day 2 proliferative responses to Mls^a and allo–H-2 stimulators both required about the same dose of stimulator cells for optimal responses (10^6); both responses then declined in parallel with lower numbers (Fig. 3A). Thus, in the case of both normal T cells and the T cell clone, the stimulator cells are of similar potency with respect to proliferation, but in neither case was PI hydrolysis stimulated with Mls^a.

We next examined PI hydrolysis in T hybridoma cells. This was of interest, since independence of IL-2 secretion from PI hydrolysis has previously been shown in a mutant TCR-negative helper hybridoma that had been reconstituted with the TCR complex by gene transfection (*10*). The T cell hybridoma we used, KW3, is a fusion product of clone K3.9 and the TCR α/β^- mutant of the murine thymoma BW5147. This hybridoma has the same pattern of Mls- and allo-reactivity as the parent clone K3.9 (*14*). IL-2 secretion by KW3 occurred in response to both allo–H-2–and Mls^a-positive stimulators (Table 1) and displayed a comparable dose dependency for the number of stimulators required. However, PI hydrolysis in KW3 was evident only in response to allo–H-2. Thus, the failure of T cells to show PI hydrolysis in response to

Fig. 2. Inositol phosphate production by dual-reactive clone K3.9 in response to allo–class II and Mls^a determinants. T cell clone K3.9 (10^6 per point) was first labeled with [^{3}H]myo-inositol and then incubated with 5×10^6 T-depleted, mitomycin C–treated spleen stimulators. Incubations were stopped at the indicated times, and [^{3}H]inositol phosphates were extracted as described (*12*). Stimulators were syngeneic B10.D2 (*H-2^d*, *Mls^b*) (■); DBA/2 (*H-2^d*, *Mls^a*) (●); and B10.P (*H-2^p*, *Mls^b*) (○). Results are mean ± SEM of triplicate samples. In a T cell proliferation assay measured on day 2, clone K3.9 gave proliferative responses to Mls^a-positive DBA/2 stimulators and to H-2–disparate B10.P stimulators, but not to syngeneic B10.D2 stimulators. Stimulators (S) were kept constant at 2×10^5 per well, and the number of responders (R) varied. At a ratio of R:S of 1:4, [^{3}H]thymidine incorporation in response to syngeneic stimulators was 600 cpm; to allo–class II, 21,700 cpm; and to Mls^a, 26,500 cpm. At R:S of 1:8, [^{3}H]thymidine incorporation was 300 cpm, 10,100 cpm, and 16,600 cpm, and at R:S of 1:16 was 200 cpm, 5,100 cpm, and 7,600 cpm, respectively. Thus, comparable cell numbers were required to achieve similar proliferative responses to both allo–class II and Mls^a antigens.

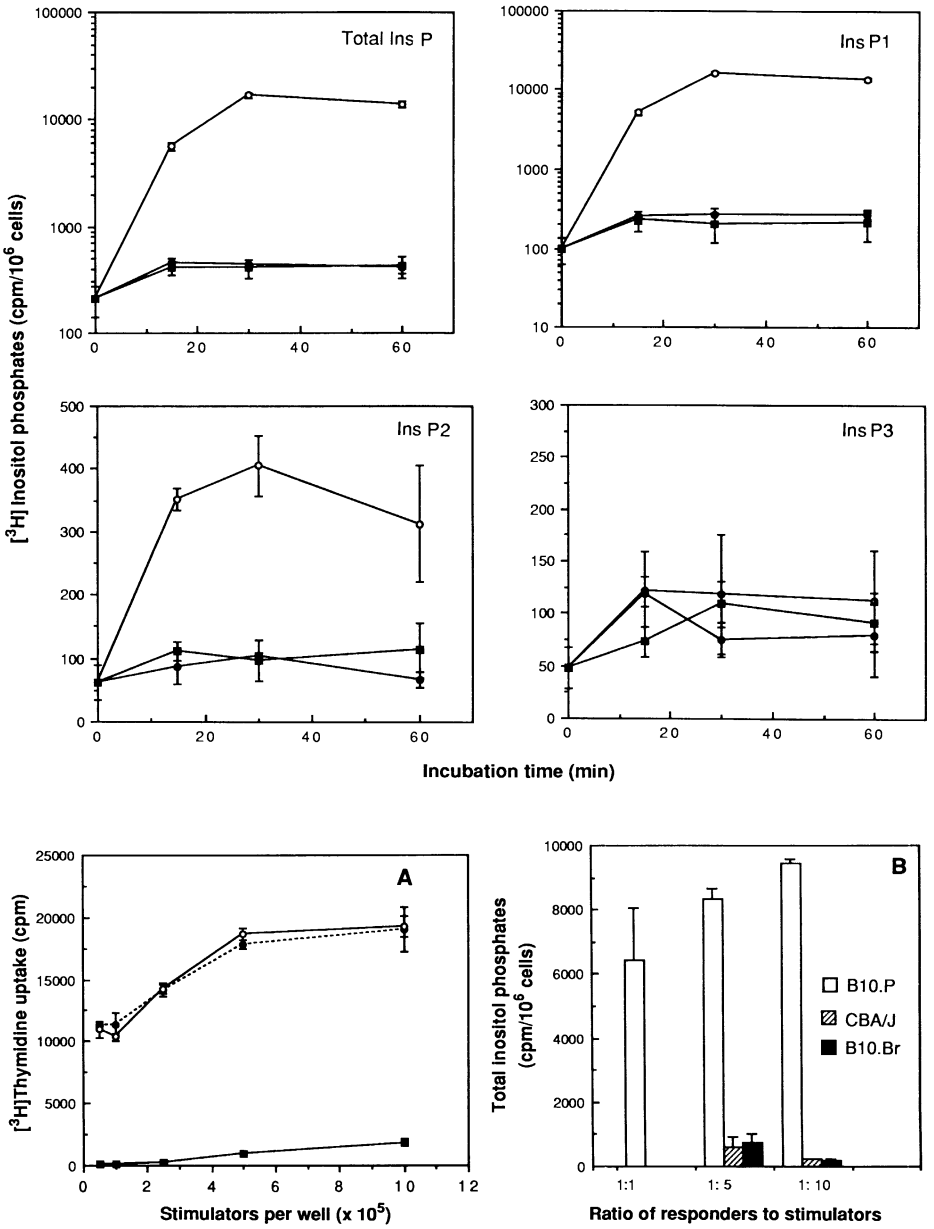

Fig. 3. Dose response of (**A**) Proliferation and (**B**) PI hydrolysis by K3.9 in response to allo–class II and Mls^a stimulation. (A) Clone K3.9 was cultured at 4×10^4 per well with 2×10^5 to 10^6 mitomycin C–treated, T-depleted spleen cells, prepared as in Fig. 1. Cells were cultured for 24 hours before wells were pulsed with 1 μCi of [^{3}H]thymidine. Cultures were harvested 18 hours later. Stimulators were B10.P (*H-2^p*, *Mls^b*) (○); CBA/J (*H-2^k*, *Mls$^{a/c}$*) (●); and B10.Br (*H-2^k*, *Mls^b*) (■). (B) Clone K3.9 (10^6 per point) was first labeled with [^{3}H]myo-inositol and then incubated with the indicated number of stimulator B cells for 45 min. Highly purified B cell stimulators were prepared as described (*14*). [^{3}H]Inositol phosphates were extracted and purified as in Fig. 2. Results are mean ± SEM of triplicate samples. Open bars, B10.P; striped bars, CBA/J; solid bars, B10.Br.

Mls^a antigens applies not only to the T cell line and clone but also to the T hybridoma.

In accounting for the failure of Mls^a antigens to elicit PI hydrolysis, one must consider the receptor:ligand interactions involved in Mls^a recognition. There are currently three models for Mls determinant recognition by T cells. One suggests that Mls determinants are presented as peptide fragments associated with class II molecules and are seen as such by responding T cells (*4, 15*). A second model suggests the existence of a unique, independent receptor for Mls determinants which, coupled with TCR–class II interaction, initiates the response (*16–18*). A third model proposes that the *Mls* gene product is a cell surface molecule that can associate with class II molecules and interact directly with certain discrete regions of the TCR V_β chain (*19*). All of these models are in accord with the view that interaction with the TCR accounts for the selective V_β gene usage observed in anti-Mls^a responses. One explanation for why PI turnover is stimulated by allo–H-2 but not Mls^a antigens is that responses to Mls^a antigens might involve relatively low-avidity interactions: the threshold TCR occupancy required for an anti-Mls response might thus be below that necessary to detect PI metabolism. Although it is difficult to measure avidity of T cell:stimulator interactions, the degree of inhibition seen with limiting concentrations of monoclonal antibodies (MAb) to CD4 and to class II does not support differences in the relative avidity of the K3.9 clone for these ligands (*20*).

Our data are not inconsistent with a model proposing the existence of a unique receptor for Mls (*16–18*); but another explanation is suggested by the observation that there are at least two different forms of the TCR-CD3 complex. In the T cell hybridomas studied, about 80% of complexes were found to have ζ-ζ disulphide-linked homodimers, whereas 20% have ζ-η heterodimers (*21*). Although both forms of the TCR complex can lead to cell triggering as manifested by IL-2 secretion (*10*) and activation-induced growth inhibition (*22*), only ζ-η–containing TCR-CD3 complexes are coupled to PI turnover (*22*). In view of this finding, it is conceivable that anti-Mls^a responses involve only ζ-ζ–containing TCR. Until Mls determinants are characterized biochemically, this possibility is difficult to address directly. IL-2 secretion (Table 1) (*10*), activation-induced growth inhibition (*22*), proliferation (Figs. 1 to 3), and target cell lysis by cytolytic T lymphocytes (*9*) can all occur in the absence of detectable activation of the PI pathway. This does not preclude a role for the PI pathway in T cell activation. It does, however, suggest that PI

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 ($H\text{-}2^k$, $Mls^{a/c}$) 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.