Brief Definitive Report

Evidence for Differential Intracellular Signaling via CD4 and CD8 Molecules

By Kodimangalam S. Ravichandran and Steven J. Burakoff

From the Division of Pediatric Oncology, Dana-Farber Cancer Institute and the Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115

Summary

Although both the CD4 and CD8 molecules enhance antigen responsiveness mediated by the T cell receptor (TCR), it is not known whether CD4 and CD8 initiate similar or different intracellular signals when they act as coreceptors. To characterize the early signals transmitted by CD4 and CD8, both CD4 and CD8α were expressed in the same murine T cell hybridoma. In the double positive transfectants, CD4 and CD8 associated with equal amounts of $p56^{lck}$ (Lck), and both molecules enhanced interleukin 2 (IL-2) production equivalently when cross-linked with suboptimal levels of anti-TCR antibody. However, in an in vitro kinase assay, cross-linking CD4 initiated fourfold greater kinase activity compared with CD8 cross-linking. In the same assay, when CD4 or CD8 was cross-linked to the TCR, novel phosphorylated proteins were found associated with the TCR/CD4 complex but not with the TCR/CD8 complex. Consistent with this data, antiphosphotyrosine immunoblotting revealed greater tyrosine phosphorylation of intracellular substrates after TCR/CD4 cross-linking compared with TCR/CD8 cross-linking. Additionally, a specific protein kinase C inhibitor (RO318220) inhibited CD8-mediated enhancement of IL-2 production far more effectively than CD4-mediated enhancement. Thus, it appears that CD8α may depend more on a protein kinase C–mediated signaling pathway, whereas CD4 may rely on greater tyrosine kinase activation. Such differential signaling via CD4 and CD8 has implications for thymic ontogeny and T cell activation.

CD4 and CD8 are nonpolymorphic cell surface glycoproteins expressed on subsets of thymocytes and mature peripheral T cells (1, 2). Generally, CD4 is expressed on Th cells that recognize Ag in association with class II MHC molecules, and CD8 is expressed on CTLs that recognize Ag in association with class I MHC molecules (1, 3, 4). CD4 and CD8 actively participate as coreceptors during T cell signaling and enhance Ag responsiveness mediated by the TCR (5–9). Although both CD4 and CD8 bind to the nonreceptor tyrosine kinase $p56^{lck}$ (Lck) (10, 11), it is not known whether CD4 and CD8 molecules initiate similar or different intracellular signals when they function as coreceptors.

Determining the signals initiated by CD4 and CD8 may be important to our understanding of thymocyte differentiation. Two models have been proposed to explain the mechanism whereby immature thymocytes differentiate from a stage where they express both CD4 and CD8 molecules ("double positive") to a mature T cell expressing either CD4 or CD8 (12, 13). The instruction model proposes that engagement of the TCR with either CD4 and CD8 during the double positive stage leads to retention of the bound coreceptor and loss of the other. This model, which is supported by several transgenic mouse studies (14–16), would seem to require differential signaling via the CD4 and CD8 molecules. The stochastic model predicts that double positive thymocytes turn off CD4 or CD8 randomly and are then selected if they express the correct TCR/coreceptor combination. This model would not require differential signaling by CD4 and CD8 and some recent studies support this model (17, 18). Delineation of the signaling pathways used by CD4 and CD8 and determining if they initiate different intracellular signals when they act as coreceptors would be crucial to the understanding of T cell development and the mechanisms of T cell activation. In this report, we address this issue of signaling by CD4 and CD8α and find evidence for differential signaling via the two molecules.

Materials and Methods

Cell Lines. BYDP, a murine T cell hybridoma expressing human CD4 and CD8α, was generated by retrovirus-mediated gene transfer of CD4 and CD8 into the By155.6 hybridoma (19, 20). The cell line expressing cytoplasmic tail–deleted CD4 (Δ13) has been previously described (21). The HLA-A2–reactive hybridoma BNK was generated by fusing BW5147 cells with splenocytes from C57BL/6 mice immunized with Jurkat cells transfected with HLA-A2. BNK-expressing human CD8 was generated by retrovirus-mediated gene transfer (20).

Assay for IL-2 Production. 96-well plates were coated with rabbit

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/94/2/0727/06 $2.00
Volume 179 February 1994 727-732

anti-mouse IgG (RAM) (5 μg/ml) overnight at 4°C, then blocked with 1% gelatin for 1 h at room temperature (RT). After washing, varying concentrations of anti-TCR mAb (F23.1) were added and incubated for 1 h at RT. Subsequently, anti-CD4 or anti-CD8 mAbs were added (1 mg/ml final) and incubated for another hour at RT. Unbound Abs were removed by washing three times with PBS, and T cell hybridomas (5×10^4/well) were added and stimulated in triplicate cultures for 24 h. The amount of IL-2 in the supernatants was assessed using the IL-2–dependent cell line CTLL-20 (19). In experiments where the effect of protein kinase C inhibitor RO318220 (Hoffman-La Roche, Nutley, NJ) was tested, the assays were performed as above in the absence or presence of the drug (2 μg/ml final) during T cell stimulation.

Stimulation of T Cell Hybridoma and Thymocytes. BYDP cells (2×10^7/ml) were incubated in 1.5-ml microfuge tubes with or without anti-TCR Ab (F23.1) and anti-CD4 (Leu3a) or anti-CD8 (Leu2a) Ab (each at 500 ng/ml final) for 10 min on ice. RAM (10 μg/ml final) was added for crosslinking and incubated for a further 10 min on ice. The cells were then incubated at 37°C for 2 min unless otherwise indicated. The cells were pelleted by a pulse spin, washed once with PBS containing 0.4 mM Na_3VO_4 and 5 mM NaF, and lysed in a buffer containing 1% Brij 96 or 0.5% Triton X-100, 50 mM Tris, pH 7.6, 150 mM NaCl, 1 mM Na_3VO_4, 10 mM NaF, 10 mM sodium pyrophosphate, 10 μg/ml each of aprotinin and leupeptin, and 2 mM PMSF. After spinning down nuclei, the lysates were collected. C57BL/6 thymocytes were isolated from 3-wk-old mice and cultured for 8 h at 37°C. The cells (5×10^6 thymocytes per sample) were then stimulated with anti-TCR Ab (H57) and/or anti-CD4 (GK1.5) or anti-CD8 (53.6.7) mAb as described above.

Immunoprecipitations and In Vitro Kinase Assays. To the lysates from 5×10^6 cell equivalents, 50 μl of a 50% solution of protein A-Sepharose beads was added with or without the addition of anti-CD4 or anti-CD8 mAb (500 ng/sample). After incubation for 2 h at 4°C, the beads were washed four times with a buffer containing 0.1% Brij 96, 20 mM Hepes, pH 7.4, 150 mM NaCl, 1 mM Na_3VO_4, 10 mM NaF, and 10 μg/ml each of aprotinin and leupeptin (immunoprecipitate (IP) wash buffer). The beads were washed once with the same buffer without the detergent and resuspended in 50 μl of kinase reaction buffer (10 mM $MnCl_2$, 5 mM Hepes, 5 mM *p*-nitrophenylphosphate, 10 μCi γ-[^{32}P]ATP, 0.1 mM Na_3VO_4, and 10 μg/ml each of aprotinin and leupeptin). The kinase reactions were performed for 2 min at 30°C and stopped by addition of 16 μl of 4× sample buffer containing 2-ME (5% final). The samples were boiled for 5 min, the proteins resolved by 6–12% SDS-PAGE, transferred to Polyvinylidene difluoride (PVDF) membranes (Millipore Corp., Bedford, MA), and visualized by autoradiography.

Antiphosphotyrosine Immunoblotting. BYDP cells were stimulated with the indicated Abs and 1.5×10^6 cell equivalents were loaded per lane and resolved by 6–10% SDS-PAGE. The proteins were transferred to nitrocellulose and immunoblotted with antiphosphotyrosine Ab 4G10 (Upstate Biotechnology Inc. [UBI], Lake Placid, NY) and developed by the enhanced chemiluminescence (ECL) system (Amersham Corp., Arlington Heights, IL). For 4G10 immunoprecipitations, 10^7 cell equivalents were incubated with 2 μg of 4G10 Ab and 50 μl of protein A-Sepharose beads (prebound with RAM) for 2 h at 4°C. After washing with IP wash buffer, the bound proteins were eluted from the beads using 10 mM *p*-nitrophenylphosphate for 30 min on ice. The eluted proteins were resolved by 6–12% SDS-PAGE and immunoblotted with antiphosphotyrosine Ab as described above.

Anti-Lck Blotting. BYDP or Δ13 cells (1.5×10^7 per sample) were lysed in Brij 96 detergent and immunoprecipitated with Leu3a, Leu2a, or isotype-matched control Ab (each at 1.25 μg per sample) and protein A–Sepharose. The proteins were resolved by 10% SDS-PAGE and immunoblotted with polyclonal anti-Lck (COOH-terminal region) Ab (UBI) and developed by ECL.

Results and Discussion

Crosslinking of CD4 and CD8 on Thymocytes Leads to Differential Activation of Lck. Thymocytes from 3-wk-old C57BL/6 mice were cultured for 8 h, then activated by crosslinking CD4 or CD8 alone or with the TCR for 2 min. at 37°C. After immunoprecipitation with protein A–Sepharose, the level of activation of Lck associated with CD4 or CD8 was assessed by an in vitro kinase assay (Fig. 1). A fourfold greater activation of Lck (assessed by in vitro autophosphorylation) was observed after CD4 crosslinking (either alone or with TCR) compared with CD8 crosslinking. It has been reported that much greater levels of Lck associate with CD4 than with CD8 in double positive thymocytes (22). However, we observed that after lysis in Brij 96 detergent, there was at most a twofold greater association of Lck with CD4 than CD8 (data not shown). Under these conditions, CD4 crosslinking induced fourfold greater kinase activity compared with CD8 crosslinking. These data suggested that in double positive thymocytes, CD4 and CD8 may initiate different signals.

Comparable Levels of Lck Association with CD4 and CD8 in the Same Murine T Cell Hybridoma. Because of the heterogeneity of thymocyte populations and the constitutively activated state of freshly isolated thymocytes, characterization of signaling by CD4 and CD8 has been technically difficult. To better delineate the early signaling cascade initiated by CD4 and CD8, a system was needed where both molecules will associate with equivalent levels of Lck within the same T cell and function comparably as coreceptors as assessed by a late activation event (e.g., IL-2 production). For this reason, we chose to coexpress CD4 and CD8α in a murine T cell hybridoma By155.16, and to evaluate signals initiated via the two molecules. By155.16 expresses a TCR that is specific for

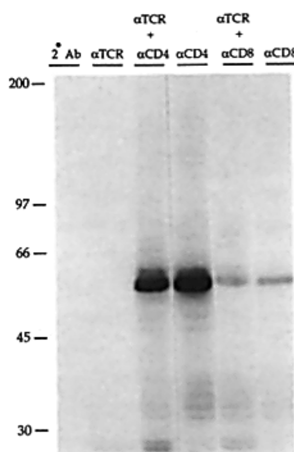

Figure 1. Effect of CD4 and CD8 crosslinking in thymocytes. Thymocytes from 3-wk-old C57BL/6 mice were isolated and stimulated with anti-TCR Ab (H57) and/or anti-L3T4 or anti-Lyt2 Abs for 2 min at 37°C, lysed using 1% Brij 96 detergent, and immunoprecipitated with protein A–Sepharose beads. In vitro kinase assays were performed for 2 min at 30°C; proteins were separated by SDS-PAGE and visualized by autoradiography.

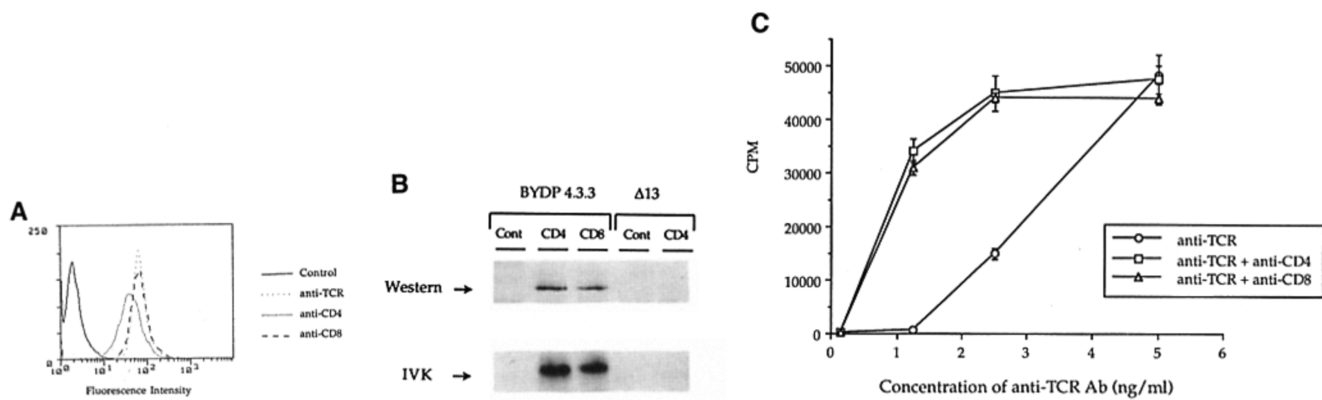

Figure 2. Characterization of CD4 and CD8 expressed in BYDP cells. (*A*) BYDP cells were stained with F23.1 (*anti-TCR*), Leu3a (*anti-CD4*), or Leu2a (*anti-CD8*) Abs, and after addition of FITC-labeled goat anti-mouse Ig as a second step reagent, the cells were analyzed by flow cytometry. (*B*) BYDP or Δ13 (hybridomas expressing cytoplasmic tail-deleted CD4) (1.5×10^7 cells per sample) were lysed in Brij 96 detergent and immunoprecipitated with anti-CD4, anti-CD8, or control IgG Abs, separated by SDS-PAGE, and immunoblotted with polyclonal anti-Lck Ab (UBI) (*top*). The basal kinase activity associated with CD4 and CD8 was measured in a parallel experiment (5×10^6 cells/sample) by an in vitro kinase assay (*bottom*). The amount of radioactivity incorporated (determined by β-scoping) showed equivalent counts in CD4 and CD8 immunoprecipitates in BYDP and background counts in control IgG or with Δ13 cells. (*C*) BYDP cells (5×10^4/well) were stimulated in triplicate in 96-well plates precoated with RAM and subsequently with different concentrations of F23.1 and 1 mg/ml of anti-CD4 or anti-CD8 Abs. The supernatants collected after 24 h were tested for the presence of IL-2 using the IL-2–dependent cell line CTLL-20. [^{3}H]Thymidine incorporation by CTLL cells during the last 4 h of a 24-h culture is plotted.

HLA-DR, and produces IL-2 in response to stimulation with cells bearing HLA-DR (19) or after Ab crosslinking of the TCR (7).

Double positive transfectants that expressed equivalent levels of human CD4 and human CD8α at the cell surface (BYDP) were generated (Fig. 2 *A*). Immunoprecipitation of CD4 or CD8 from these cells and immunoblotting for Lck demonstrated that both molecules associated with equivalent amounts of Lck (Fig. 2 *B*). An in vitro kinase assay after CD4 or CD8 immunoprecipitation showed that equivalent levels of basal kinase activity were associated with CD4 and CD8 (Fig. 2 *B*).

CD4 and CD8 Function Equivalently as Coreceptors for IL-2 Production. If T cells are stimulated by suboptimal concentrations of anti-TCR Ab, crosslinking CD4 or CD8 to the TCR will enhance activation (5, 7). When double positive transfectants were stimulated with suboptimal levels of anti-TCR Ab, crosslinking of either CD4 or CD8 enhanced IL-2 production equivalently (5–20-fold compared with crosslinking the anti-TCR Ab alone) (Fig. 2 *C*). These results were confirmed with several independently derived double positive hybridomas. These data indicated that both molecules were able to provide equivalent coreceptor function for IL-2 production.

Differential Effects of TCR/CD4 vs. TCR/CD8 Crosslinking. The level of Lck activation after CD4 or CD8 crosslinking in the double positive hybridoma was assessed by an in vitro kinase assay. After TCR crosslinking alone, or after crosslinking CD4 or CD8 to the TCR for 2 min at 37°C, cells were lysed in Brij 96, a mild detergent that has been shown to preserve molecular complexes associated with TCR, CD4, or CD8 (23). Ab crosslinking of CD4 resulted in fivefold greater autophosphorylation of Lck compared with CD8 crosslinking (Fig. 3 *A*). Thus, even though the double positive hybridomas have comparable amounts of Lck bound to CD4 and CD8, crosslinking CD4 initiated greater Lck kinase activity than crosslinking CD8. We also noted several novel phosphorylated bands that appeared only after TCR/CD4 crosslinking, but not after TCR/CD8 crosslinking, nor after crosslinking CD4 alone. Even after prolonged exposure of the autoradiographs, associated bands were not detected in lanes from TCR/CD8–crosslinked cells (Fig. 3 *A*). These data suggest that even though both CD4 and CD8 can enhance IL-2 production equivalently, TCR/CD4 and TCR/CD8 crosslinking may initiate different signals.

The novel bands detected in TCR/CD4 lanes could be due to the association of new proteins during TCR/CD4 activation, or results from their association with CD4 or TCR/CD3 chains after lysis. To distinguish between these two alternatives, anti-CD4 Ab was added to all samples after activation and detergent lysis. Except for the basal Lck kinase activity that was precipitated by the anti-CD4 Ab (seen in all lanes), no novel bands appeared in TCR alone or in TCR/CD8–crosslinked lanes. This suggests that the novel bands reflect proteins that associated with TCR/CD3 and/or CD4 chains only after activation. Post-lysis addition of anti-CD8 Ab did not result in any novel bands (data not shown). To rule out the possibility that these results were unique to the Abs that were used, a panel of anti-CD4 and anti-CD8 Abs was tested. Markedly enhanced Lck activity and novel associated bands were seen with all anti-CD4 mAbs used (Leu3a, OKT4C, and OKT4D), but with neither of the anti-CD8 mAbs used (Leu2a and OKT8) (data not shown). T cell hybridomas expressing CD4 or CD8 alone revealed patterns of phosphorylation similar to that seen in the double positive hybridomas (after CD4 or CD8 crosslinking alone or with TCR) (data not shown). Thus, these results do not appear to be unique to double positive cells but rather reflect an inherent property of CD4 or CD8 signaling. Transfectants expressing a

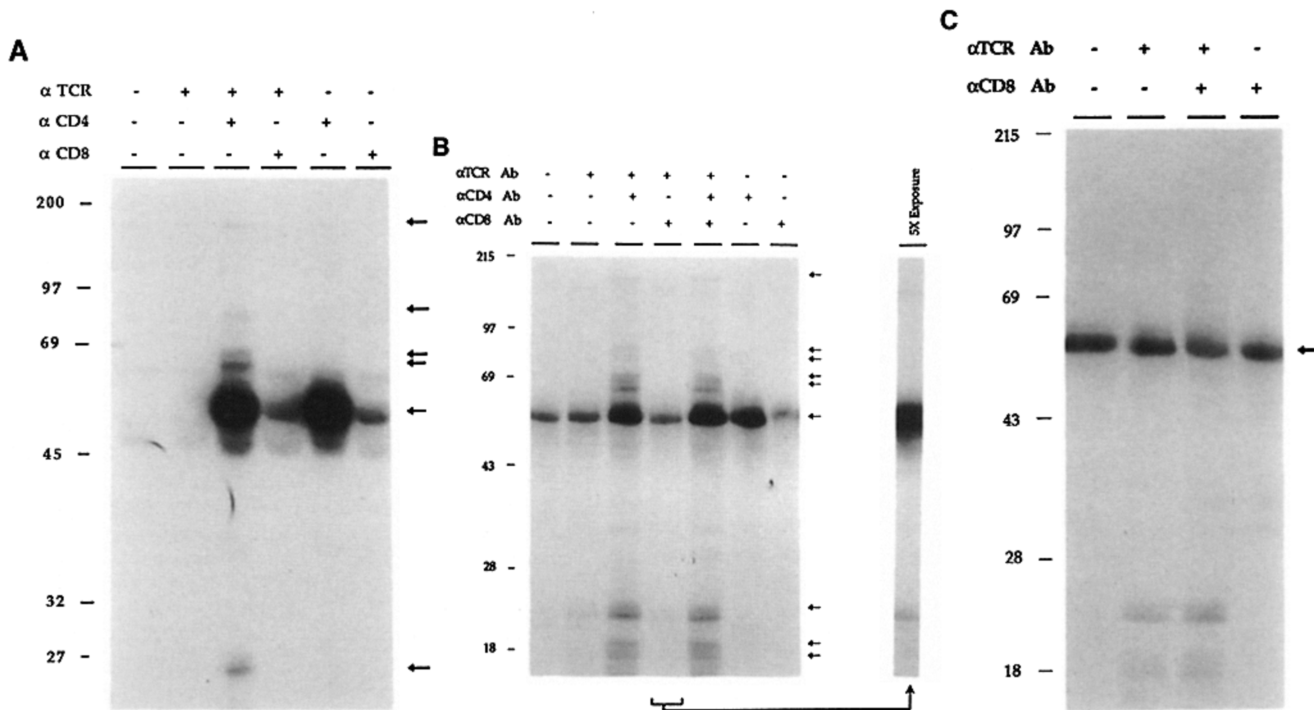

Figure 3. Differential activation of Lck after CD4 or CD8 crosslinking and association of novel proteins after TCR/CD4 crosslinking. (*A*) BYDP cells were stimulated with the indicated Abs for 2 min at 37°C, lysed in 1% Brij-96, and immunoprecipitated with protein A–Sepharose beads without any further addition of antibodies. In vitro kinase assays were performed, and the proteins resolved by a 6–12% SDS-PAGE and visualized by autoradiography. (*Left*) Molecular weight standards (*arrows, right*) bands that were different after CD4 and CD8 crosslinking. β-scoping indicated fourfold greater radioactivity incorporated in the 56-kD band in TCR/CD4 crosslinked lanes compared with TCR/CD8 crosslinking, and a 1.5-fold increase after TCR/CD8 crosslinking compared with CD8 crosslinking alone. (*B*) BYDP cells were stimulated and lysed as in *A*. After lysis, anti-CD4 Ab (500 ng/sample) was added to all samples and immunoprecipitated with protein A–Sepharose. In vitro kinase assays and autoradiography were performed as above. Radioactive counts in the 56-kD band were fourfold higher in TCR/CD4 or CD4 crosslinked lanes compared with TCR/CD8 lanes. (*Arrows*) Bands that appear in the TCR/CD4 but not in TCR/CD8 lanes. Fivefold longer exposure of the TCR/CD8 lane is shown on an adjacent panel. Even after this prolonged exposure, the 85- and 190-kD bands were seen only in the TCR/CD4 lanes. (*C*) BNK-CD8 cells were stimulated with the indicated Abs and lysed as above. 500 ng anti-CD8 Ab was added to all samples after lysis, and immunoprecipitation and in vitro kinase assays were performed as above. Radioactivity incorporated in the 56-kD band was equivalent in all four lanes.

cytoplasmic tail–deleted CD4 (21) did not show the phosphorylation pattern of wild-type CD4, indicating that elements within the cytoplasmic domain of CD4 are necessary for the observed results (data not shown).

It is possible that the lower phosphorylation seen after CD8 crosslinking may be due to the activation of a phosphatase by CD8 that downregulated phosphorylation. If this was the case, then simultaneous crosslinking of CD4 and CD8 to the TCR would lead to a pattern of phosphorylation similar to TCR/CD8 crosslinking. As shown in Fig. 3 *B* (lane *5*), simultaneous crosslinking of CD4 and CD8 to the TCR resulted in a phosphorylated pattern identical to TCR/CD4 crosslinking. Though this does not formally rule out the activation of a phosphatase by CD8 crosslinking, it seems likely that CD4 crosslinking initiates greater kinase activity compared with CD8.

Since the TCR of the BYDP hybridoma is reactive with human class II MHC molecules, it is possible that the enhanced activation of Lck and association of new substrates with CD4 compared with CD8 may have been skewed by the specificity of the TCR. To assess this possibility, we derived an HLA-A2–reactive hybridoma (BNK) and generated transfectants expressing human CD8. The human CD8 molecule expressed in BNK was fully functional as assessed by its association with Lck and by enhancement of IL-2 production when crosslinked to the TCR (data not shown). In vitro kinase assays performed after TCR/CD8 crosslinking or CD8 crosslinking alone resulted in minimal enhancement over the basal Lck kinase activity (Fig. 3 *C*). Several weakly phosphorylated bands were observed after TCR/CD8 crosslinking in BNK, however, the major phosphorylated bands seen after TCR/CD4 crosslinking were absent. Thus, it seems unlikely that the specificity of the TCR influences coreceptor signaling.

To determine if the enhanced activation of Lck after TCR/CD4 crosslinking, seen in vitro kinase assays, also resulted in greater phosphorylation of intracellular substrates in vivo, we compared the pattern of tyrosine phosphorylated proteins that appear after TCR/CD4 vs. TCR/CD8 crosslinking by immunoblotting with an antiphosphotyrosine Ab (4G10). Analysis of total cell lysates with 4G10 showed that, whereas TCR/CD8 crosslinking resulted in a marginal increase in tyrosine phosphorylation of intracellular substrates,

TCR/CD4 crosslinking gave significantly enhanced tyrosine phosphorylation of several substrates (Fig. 4 *A*). The possibility that CD4 and CD8 crosslinking might enhance tyrosine phosphorylation with different kinetics was ruled out by performing a time course experiment to determine the appearance of tyrosine phosphorylated substrates (Fig. 4 *A*). Many of the bands that were enhanced by TCR, TCR/CD4, or TCR/CD8 crosslinking peaked by 1 min but started to disappear after 5 min. Enhancement of tyrosine phosphorylated proteins by TCR/CD4 crosslinking was more clearly demonstrated by immunoprecipitation with an antiphosphotyrosine Ab followed by release of proteins bound to 4G10 by *p*-nitrophenylphosphate, resolution by SDS-PAGE, and immunoblotting with 4G10. (Fig. 4 *B*).

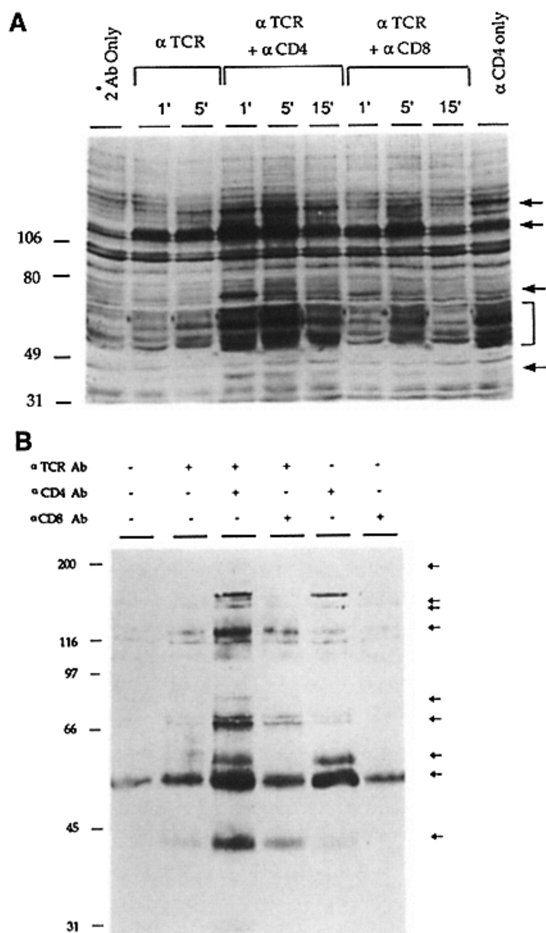

Figure 4. Comparison of in vivo tyrosine phosphorylated substrates after TCR/CD4 or TCR/CD8 crosslinking. (*A*) BYDP cells (5 × 10^6/0.5 ml) were stimulated with the indicated Abs for 1, 5, or 15 min. After washing, the cells were lysed using 0.5% Triton X-100. Lysates from 1.5 × 10^6 cell equivalents were loaded per lane and resolved by 6–10% SDS-PAGE. After transfer to nitrocellulose, the tyrosine-phosphorylated proteins were revealed using antiphosphotyrosine Ab 4G10 and ECL. (*Left*) Migration of molecular weight standards, (*arrows, right*) prominent bands appearing with TCR/CD4 crosslinking. (*B*) BYDP cells (10^7) were stimulated and immunoprecipitated with antiphosphotyrosine Ab 4G10 and protein A–Sepharose beads. After washing extensively, the bound proteins were eluted from the beads using 10 mM *p*-nitrophenylphosphate for 30 min on ice. The eluted proteins were resolved by 6–12% SDS-PAGE and immunoblotted with antiphosphotyrosine Ab. (*Left*) Migration of molecular weight standards; (*arrows, right*) prominent bands appearing with TCR/CD4 crosslinking.

CD8-mediated, but Not CD4-mediated Enhancement of IL-2 Production Is Sensitive to a Protein Kinase C Inhibitor. Tyrosine kinase activation is the earliest known signaling event following TCR activation. If the initial onset of tyrosine kinase activation is inhibited by drugs such as herbimycin or genistein, subsequent IL-2 production is prevented (24). To determine if TCR/CD8 signaling may depend more on a pathway involving protein kinase C, the effect of a specific protein kinase C inhibitor, RO318220, on enhancement of IL-2 production by CD4 and CD8, was evaluated (Fig. 5). RO318220 significantly inhibited the enhancement of IL-2 production by TCR/CD8 crosslinking, whereas TCR/CD4–mediated IL-2 enhancement was far less affected. Thus, it appears that CD8 may depend more on a protein kinase C–mediated signaling pathway whereas CD4-mediated signaling may have a greater dependence on activation of tyrosine kinases. This is consistent with the observation of O'Rourke and Mescher (25) that the upregulation of CD8 avidity for class I MHC, while dependent on initial tyrosine kinase activity, is sensitive to staurosporine. In addition, the interaction of CD8 with MHC class I also leads to enhanced IP3 generation (25).

In conclusion, CD4 and CD8 crosslinking may initiate different signals in both thymocytes and in a $CD4^+CD8^+$ T cell hybridoma. Julius et al. (26) have also proposed that $CD4^+$ and $CD8^+$ T cells signal differently. Although both molecules depend initially on tyrosine kinase activation, CD8 may depend more on a protein kinase C–mediated signaling pathway, whereas CD4 may rely on greater tyrosine kinase activation. Such differential signaling may provide a mechanism for instructing double positive thymocytes to mature into $CD4^+$ or $CD8^+$ single positive thymocytes. It cannot be ruled out, however, that double positive thymocytes differentiate stochastically to single positive thymocytes and subsequently rely on differential signaling of CD4 vs. CD8.

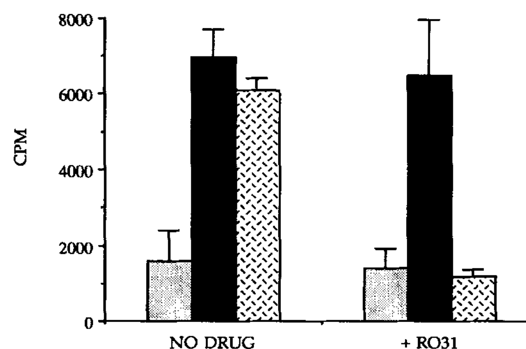

Figure 5. Effect of protein kinase C inhibitor on IL-2 production. BYDP cells were stimulated as described in Fig. 2 *C* with or without 2 μg/ml of RO318220, and the supernatants were tested for the presence of IL-2 on CTLL-20, as in Fig. 2 *C*. (▩) Anti-TCR; (■) anti-TCR + anti-CD4; and (▨) anti-TCR + anti-CD8.

Since only CD8α was expressed in the double positive hybridoma, it will be important to determine the contribution of CD8β to early signaling events. Also, identification of the substrates that associate with CD4, as well as further delineation of CD4 and CD8 signaling pathways, should lead to a better understanding of thymic ontogeny and T cell activation.

We thank Dr. Georg Hollander for the CD4 and CD8 retrovirus producer lines and for initial work on this project. We thank Vivien Igras for generating the BNK hybridoma, and we thank Dr. Ulrike Lorenz for helpful discussions.

This work was supported by National Institutes of Health grant AI-17528 (to S. J. Burakoff) and a grant from F. Hoffmann-LaRoche.

Address correspondence to Dr. Kodimangalam S. Ravichandran, DANA 1722, Division of Pediatric Oncology, Dana-Farber Cancer Institute, 44, Binney St., Boston, MA 02115.

Received for publication 11 August 1993 and in revised form 28 October 1993.

References

1. Bierer, B.E., B.P. Sleckman, S.E. Ratnofsky, and S.J. Burakoff. 1989. The biological roles of CD2, CD4, and CD8 in T cell activation. *Annu. Rev. Immunol.* 7:579.
2. Parnes, J.R., 1989. Molecular biology and function of CD4 and CD8. *Adv. Immunol.* 44:265.
3. Doyle, C., and J.L. Strominger. 1987. Interaction between CD4 and class II MHC molecules mediates cell adhesion. *Nature (Lond.).* 330:526.
4. Rosenstein, Y., S. Ratnofsky, S.J. Burakoff, and S.H. Herrmann. 1989. Direct evidence for binding of CD8 to HLA class I antigens. *J. Exp. Med.* 169:149.
5. Janeway, C.A. 1991. The co-receptor function of CD4. *Sem. Immunol.* 3:153.
6. Glaichenhaus, N., N. Shastri, D.R. Littman, and J.M. Turner. 1991. Requirement for association of $p56^{lck}$ with CD4 in antigen-specific signal transduction in T cells. *Cell.* 64:511.
7. Collins, T.L., S. Uniyal, J. Shin, J.L. Strominger, R.S. Mittler, and S.J. Burakoff. 1992. $p56^{lck}$ association with CD4 is required for the interaction between CD4 and the TCR/CD3 complex and for optimal antigen stimulation. *J. Immunol.* 148: 2159.
8. Ratnofsky, S., A. Peterson, J. Greenstein, and S.J. Burakoff. 1987. Expression and function of CD8 in a murine T cell hybridoma. *J. Exp. Med.* 166:1747.
9. Chaulpny, N.J., J. Ledbetter, and P. Kavathas. 1991. Association of CD8 with $p56^{lck}$ is required for early T cell signalling events. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:1201.
10. Rudd, C., J. Trevillyan, J. DasGupta, L. Wong, and S. Schlossman. 1988. The CD4 receptor is complexed in detergent lysates to a protein-tyrosine kinase (pp58) from human T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 85:5190.
11. Veillette, A., M.A. Bookman, E.M. Horak, and J.B. Bolen. 1988. The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine kinase $p56^{lck}$. *Cell.* 53: 301.
12. von Boehmer, H. 1986. The selection of the alpha, beta heterodimeric T cell receptor for antigen. *Immunol. Today.* 7:333.
13. von Boehmer, H. 1993. Lymphocyte lineage commitment: instruction versus selection. *Cell.* 73:207.
14. Teh, H.S., P. Kisielow, B. Scott, H. Kishi, Y. Uematsu, H. Bluthman, and H. von Boehmer. 1989. Thymic major histocompatibility complex antigens and the alpha beta T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature (Lond.).* 335:229.
15. Kaye, J., M.L. Hsu, M.E. Sauron, S.C. Jameson, N.R. Gascoigne, and S.M. Hedrick. 1989. Selective development of $CD4^+$ T cells in transgenic mice expressing a class II MHC–restricted antigen receptor. *Nature (Lond.).* 341:746.
16. Robey, E.A., B.J. Fowlkes, J.W. Gordon, D. Kioussis, H. von Boehmer, and R. Axel. 1991. Thymic selection in CD8 transgenic mice supports an instructive model for commitment to a CD4 or CD8 lineage. *Cell.* 64:99.
17. Chan, S.H., D. Cosgrove, C. Waltzinger, C. Benoist, and D. Mathis. 1993. Another view of the selective model of thymocyte selection. *Cell.* 73:225.
18. Davis, C.B., N. Killeen, M.E. Casey Crools, D. Raulet, and D.R. Littman. 1993. Evidence for a stochastic mechanism in the differentiation of mature subsets of T lymphocytes. *Cell.* 73:237.
19. Sleckman, B.P., A. Peterson, W.K. Jones, J.A. Foran, J. Greenstein, B. Seed, and S.J. Burakoff. 1987. Expression and function of CD4 in a murine T cell hybridoma. *Nature (Lond.).* 328:351.
20. Hollander, G.A., B.D. Lusky, D.A. Williams, and S.J. Burakoff. 1992. Functional expression of human CD8 in fully reconstituted mice after retroviral-mediated gene transfer of hemopoietic stem cells. *J. Immunol.* 149:438.
21. Sleckman, B.P., B.A. Peterson, A. Foran, J.C. Gorga, C.J. Kara, J.L. Strominger, S.J. Burakoff, and J.L. Greenstein. 1988. Functional analysis of a cytoplasmic domain-deleted mutant of the CD4 molecule. *J. Immunol.* 141:49.
22. A. Veillette, J.C., Zúñiga-Pflücker, J.B. Bolen, and A.M. Kruisbeek. 1989. Engagement of CD4 and CD8 expressed on immature thymocytes induces activation of intracellular tyrosine phosphorylation pathways. *J. Exp. Med.* 170:1671.
23. Beyers, A.D., L.L. Spruyt, and A.F. Williams. 1992. Molecular associations between the T-lymphocyte antigen receptor complex and the surface antigens CD2, CD4 or CD8 and CD5. *Proc. Natl. Acad. Sci. USA.* 89:2945.
24. June, C.H., M.C. Fletcher, J.A. Ledbetter, G.L. Schieven, J.N. Siegel, A.F. Phillips, and L.E. Samelson. 1990. Inhibition of tyrosine phosphorylation prevents T cell receptor–mediated signal transduction. *Proc. Natl. Acad. Sci. USA.* 87:7722.
25. O'Rourke, A., and M.F. Mescher. 1992. Cytotoxic T-lymphocyte activation involves a cascade of signaling and adhesion events. *Nature (Lond.).* 358:253.
26. Julius, M., Maroun, C., and L. Haughn. 1993. Distinct roles for CD4 and CD8 coreceptors in antigen receptor signaling. *Immunol. Today.* 14:177.