Protein tyrosine kinases are critical for the function of CD28 in T cells. We examined whether the tyrosine kinases Pyk2 and Fak (members of the focal adhesion kinase family) are involved in CD28 signaling. We found that ligating CD28 in Jurkat T cells rapidly increases the tyrosine phosphorylation of Pyk2 but not of Fak. Paxillin, a substrate for Pyk2 and Fak, was not tyrosine-phosphorylated after CD28 ligation. CD28-induced tyrosine phosphorylation of Pyk2 was markedly reduced in the absence of external Ca2+. Previous studies have shown that the T cell antigen receptor (TCR) induces tyrosine phosphorylation of Pyk2. In this report, the concurrent ligation of CD28 and TCR increased tyrosine phosphorylation of Pyk2; however, the extent of phosphorylation by both receptors was equivalent to the sum of that induced by each receptor alone. The Syk/Zap inhibitor piceatannol blocked CD28, and TCR induced tyrosine phosphorylation of Pyk2, suggesting that Syk/Zap is involved in Pyk2 phosphorylation. In contrast, the phosphatidylinositol 3-kinase inhibitor wortmannin blocked TCR- but not CD28-induced phosphorylation of Pyk2, suggesting that CD28 and TCR activate distinct pathways to induce tyrosine phosphorylation of Pyk2. Notably, depleting phorbol 12-myristate 13-acetate-sensitive protein kinase C did not block CD28- and CD3-induced tyrosine phosphorylation of Pyk2. These data provide evidence for the involvement of Pyk2 in the CD28 signaling cascade and suggest that neither Fak nor paxillin is involved in the signaling pathways of CD28.

CD28 is a 44-kDa homodimer surface glycoprotein expressed by most mature T cells (1–3). This molecule is critical for T cell activation. Thus, mice lacking CD28 because of targeted gene disruption show significant immune system defects consistent with disrupted T cell function (4, 5). Furthermore, CD28-initiated signals regulate T cell receptor (TCR)-mediated activation, as TCR-induced T cell proliferation and cytokine release are markedly reduced in the absence of CD28 ligation (2, 3).

The molecular mechanisms by which CD28 augments TCR function are not clear. Recent studies, however, have shown that CD28 and the TCR complex initiate distinct intracellular signals (2, 3). For example, TCR-induced IL-2 production is almost completely inhibited by cyclosporin A, yet this drug has no effect on the production of IL-2 by the ligation of CD28 in conjunction with phorbol 12-myristate 13-acetate (PMA) (6–8). These data, together with the fact that CD28 but not TCR induces the tyrosine phosphorylation of the adaptor protein Dok (9–13), support the notion that TCR and CD28 induce biochemically distinct intracellular signals.

Protein tyrosine kinases (PTKs) are activated after the ligation of various receptors, including TCR and CD28 (14–19). Ligating TCR rapidly activates the Src PTKs Fyn and Lck, leading to the phosphorylation of the immunoreceptor tyrosine-based activation motifs in the CD3 complex (16, 18–20). Phosphorylated immunoreceptor tyrosine-based activation motifs interact with the Src homology (SH) 2 domains of the Syk/Zap family PTKs, leading to the activation of these PTKs and in turn to the phosphorylation of an array of cellular proteins (16, 18–21). Similarly, CD28 signaling pathways involve the phosphorylation of several proteins on tyrosine residues (11–13, 22–28). Indeed, such phosphorylations are critical for CD28 function (12, 23). The cytoplasmic region of CD28 becomes rapidly tyrosine-phosphorylated after CD28 ligation, presumably by Fyn and Lck (24, 29). Tyrosine-phosphorylated CD28 associates with Src homology (SH)2-containing proteins including Itk (a member of the Tec family), phosphatidylinositol 3-kinase (PI 3-kinase), and growth factor receptor-bound protein 2 (Grb2) (22, 27, 29–30, 35). CD28-Grb2 association may link CD28 signaling pathways to Ras and in turn to downstream events such as mitogen-activated protein (MAP) kinase (36, 37). Recent studies suggest that CD28 signaling pathways also involve the activation of Rho family GTPases. For example, CD28 ligation induces tyrosine phosphorylation and activation of Vav, a guanine nucleotide exchange factor for Rho family GTPases (13, 22, 35, 36), and promotes the formation of focal adhesion-like sites where these GTPases accumulate (38). Rho family GTPases are implicated in regulating the activation of the MAP kinase c-Jun NH2-terminal kinase (JNK) (37, 39, 40). Accordingly, JNK activation has been reported to be regulated by signals initiated after CD28 aggregation (41).

Recently, a new family of PTKs has been identified as the focal adhesion PTK family. This family consists of the non-receptor, proline-rich PTKs Fak (Focal Adhesion Kinase) and Pyk2 (Proline-rich tyrosine kinase 2, also designated CAKβ, RAFTK, FAK2, or CADTK) (42–48). These kinases have a molecular mass of 110–125 kDa and are closely related in their overall structures. Fak is expressed in almost all tissues, whereas Pyk2 is expressed mainly in the central nervous system and in cells and tissues derived from hematopoietic lineages. Although in adherent cells Fak is localized to focal adhesion sites, Pyk2 is mainly diffused throughout the cyto-
plasm (49, 50). Fak and Pyk2 become tyrosine-phosphorylated and activated after the stimulation of various receptors including TCR (44, 45, 47, 51–64), and both kinases have been linked to the signaling pathways that regulate MAP kinases (45, 48, 58, 61, 59).

In the present study, we examined the involvement of Fak/Pyk2 in the CD28 signaling pathways. We found that Pyk2, but not Fak or its substrate paxillin, is rapidly tyrosine-phosphorylated and activated after CD28 ligation. We also found that simultaneously ligating CD28 and TCR had an additive effect on tyrosine phosphorylation of Pyk2. Further analysis of CD28- and TCR-induced tyrosine phosphorylation of Pyk2 showed that CD28 appears to utilize signaling pathways to phosphorylate Pyk2 distinct from those used by TCR.

EXPERIMENTAL PROCEDURES

Reagents—RPMI was purchased from Mediatech Cellgro (Herndon, VA), and EMEM was purchased from BioWhittaker (Walkersville, MD). Fetal calf serum was purchased from HyClone Laboratories, Inc. (Logan, UT). Antibiotic/antimycotic mixture and glutamine were from Life Technologies, Inc. Aprotinin, picatannol, phenylmethylsulfonyl fluoride, FMA, protease-free bovine serum albumin (BSA), protein A-agarose beads, and ω-sulfogalactosylceramide were from Sigma. LumiGLO chemiluminescence substrate kit was purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD). Polyvinylidene fluoride membranes were from Millipore (Bedford, MA). Chelythrynine chloride was obtained from Biomol (Plymouth Meeting, PA).

Antibodies—Anti-human CD28 mAb (Leu-28, clone L293) was purchased from Becton Dickinson (San Jose, CA). This anti-CD28 mAb does not induce IL-2 production from Jurkat T cells, but it significantly enhances IL-2 release induced by the combination of PMA and Ca²⁺ ionophore. Anti-human CD3 mAb was from Ancell (Bayport, MN). Anti-phosphotyrosine mAb PY-20, anti-Pyk2 mAb, anti-paxillin mAb, and anti-human CD3 mAb were from Ancell (Bayport, MN). Anti-CD28 mAb for the indicated time. Tyrrosine-phosphorylated proteins were detected as in A. Arrows indicate proteins that become tyrosine-phosphorylated after CD28 ligation.

protein kinase C (PKC), cells were incubated for 16 h at 37 °C with 400 μg/ml NMA. After washing, the cells were resuspended in 0.01% BSA/RPMI and stimulated with anti-CD3 mAb or anti-CD28 mAb as above. Immunoprecipitation and Immunoblotting—Cell lysates from 8 × 10⁶ cells were preclarified by incubating for 1 h with 10 μg of rabbit anti-mouse immunoglobulin that had been preclarified for 2 h with 50 μg of protein A-agarose. Meanwhile, the primary antibody was added to 10 μg of rabbit anti-mouse immunoglobulin that had been preclarified for 2 h at 4 °C with 50 μl of protein A-agarose beads. Preclarified lysates were then added to the protein A-antibodies mixture and incubated for 2 h at 4 °C. After incubation, the beads were pelleted by centrifugation and washed 5 times with ice-cold solubilization buffer. After the final centrifugation, the beads were resuspended in 2× SDS-polyacrylamide gel electrophoresis sample buffer (final concentration, 75 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol) and boiled for 5 min. For immunoblotting, aliquots from whole cell lysates (WCL) or immunoprecipitates were separated by SDS-polyacrylamide gel electrophoresis (10%) and electrotransferred onto polyvinylidene fluoride membranes. Immunoblotting with the horseradish peroxidase-conjugated anti-phosphotyrosine antibody PY-20 was as described previously (62, 66, 67). To confirm similar loading of samples, antibodies were stripped from the membranes as previously reported (66), and the proteins were reprobed with specific antibodies followed by horseradish peroxidase-coupled rabbit anti-mouse immunoglobulin (1:50,000 dilution). The signals were visualized using the LumiGLO kit according to the manufacturer’s recommendations.

RESULTS

Induction of Pyk2 Tyrosine Phosphorylation by Aggregating CD28—To examine whether the PTKs Pyk2 and Fak are involved in CD28 signaling, Jurkat T cells were treated with anti-CD28 mAb, and the immunoprecipitates of Pyk2 and Fak were transferred to membranes and probed with anti-phosphotyrosine mAb. Immunoblotting of WCL with anti-phosphotyrosine mAb showed that aggregating CD28 on Jurkat T cells induces tyrosine phosphorylation of several proteins, the most prominent of which were 42–46, 65, 78-, and 95-kDa proteins (Fig. 1A). In contrast, NMG did not induce detectable protein tyrosine phosphorylation. CD28-induced tyrosine phosphorylation was apparent within 2 min, peaked at 5 min, and decreased slowly after that (Fig. 1B). As shown in Fig. 2A, treating the cells with 1 or 3 μg/ml anti-CD28 mAb increased tyrosine phosphorylation of Pyk2. No detectable increase in the tyrosine phosphorylation of the kinase was seen in cells treated with NMG (Fig. 2A). Time course studies showed that the phosphorylation of Pyk2 by CD28 cross-linking was first apparent within 2 min, reached a peak by 10 min, and decreased at 20 min (Fig. 2B). In contrast, incubating the cells with anti-CD28 mAb did not induce any detectable tyrosine phosphorylation of Fak (Fig. 2C). Fak tyrosine phosphorylation was

FIG. 1. Induction of protein tyrosine phosphorylation by aggregating CD28. A, Jurkat T cells were treated with 3 μg/ml NMG or anti-CD28 mAb for 5 min at 37 °C. Proteins in cell lysates were separated by SDS-polyacrylamide gel electrophoresis, transferred to membranes, and immunoblotted with anti-phosphotyrosine mAb (α-PY). Stim., stimulus. B, Jurkat T cells were stimulated with 3 μg/ml anti-CD28 mAb for the indicated time. Tyrosine-phosphorylated proteins were detected as in A. Arrows indicate proteins that become tyrosine-phosphorylated after CD28 ligation.

Tyrosine Phosphorylation of Pyk2 by CD28 Ligation

2 M. Tsuchida, S. J. Knechtle, and M. M. Hamawy, unpublished results.
Tyrosine Phosphorylation of Pyk2 by CD28 Ligation

CD28 ligation induces tyrosine phosphorylation of Pyk2 but not of Fak or paxillin. Jurkat T cells were treated with 1 or 3 μg/ml NMG or anti-CD28 mAb for 5 min at 37°C. Pyk2 was immunoprecipitated from 8 × 10⁶ cells and analyzed by immunoblotting with anti-phosphotyrosine mAb (α-PY, upper panel) or with anti-Pyk2 mAb (lower panel). Stim., stimulus. B–D, Jurkat T cells were stimulated with 3 μg/ml anti-CD28 mAb for the indicated time at 37°C. Cell lysates were immunoprecipitated with anti-Pyk2 mAb (B), anti-Fak mAb (C), or anti-paxillin mAb (D). Immunoprecipitates were analyzed by immunoblotting with anti-phosphotyrosine mAb (α-PY, upper panel) or with the specific mAb (lower panel).

Concurrent Ligation of CD28 and TCR Leads to Additive Tyrosine Phosphorylation of Pyk2—It is now well established that signals from TCR and CD28 cooperate in the activation of T cells. Previous studies have shown that cross-linking CD28 increases the tyrosine phosphorylation of paxillin (Fig. 2D). These data show that Pyk2 is involved in CD28 signaling and suggest that neither Fak nor paxillin is involved in the signaling pathways of CD28.

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Optimal CD28-induced Pyk2 Tyrosine Phosphorylation Requires Ca²⁺—The engagement of CD28 has been shown to increase the levels of intracellular Ca²⁺ (8); thus, we examined the role of Ca²⁺ in CD28-induced tyrosine phosphorylation of Pyk2 by stimulating the cells with anti-CD28 in the presence or absence of external Ca²⁺. As shown in Fig. 4A, tyrosine phosphorylation of several proteins induced with anti-CD28 mAb or anti-CD3 mAb was profoundly reduced or completely abolished in the absence of Ca²⁺. As previously reported, anti-CD3 mAb-induced tyrosine phosphorylation of Pyk2 was reduced by Ca²⁺ depletion (Fig. 4B) (52). CD28-induced Pyk2 tyrosine phosphorylation was also reduced in the absence of Ca²⁺ (Fig. 4B).

Whether the incomplete inhibition of receptor-mediated Pyk2 tyrosine phosphorylation is due to the release of Ca²⁺ from intracellular stores or due to the tyrosine phosphorylation of Pyk2 by mechanisms that do not require Ca²⁺ awaits further investigation. Nonetheless, these results show that optimal tyrosine phosphorylation of Pyk2 by CD28 signaling requires Ca²⁺.

The Syk/Zap Inhibitor Piceatannol Blocks Both TCR and CD28-induced Tyrosine Phosphorylation of Pyk2—Syk and the related PTK Zap are critical for TCR function; however, their
role in CD28 signaling is not clear. Syk has been shown to be critical for FcεRI-mediated tyrosine phosphorylation of Pyk2 in mast cells (where FcεRI is high affinity IgE receptor) (51). Thus, we used the specific Syk/Zap inhibitor piceatannol (70) to examine the role of these PTKs in CD28- and TCR-induced Pyk2 tyrosine phosphorylation. As shown in Fig. 5A, pretreating the cells with piceatannol reduced tyrosine phosphorylation of several proteins induced by CD28 or TCR. This is not surprising, as Syk/Zap become activated early in signaling cascades. Interestingly, piceatannol inhibited both CD28- and TCR-induced tyrosine phosphorylation of Pyk2 (Fig. 5B). Such inhibition was evident at concentrations that have been reported previously to selectively influence Syk/Zap activity (70). Furthermore, piceatannol appears to be more effective in inhibiting CD28-induced tyrosine phosphorylation of Pyk2 than inhibiting CD3-induced Pyk2 phosphorylation. Although these data suggest that Syk/Zap are involved in TCR- and CD28-induced tyrosine phosphorylation of Pyk2; the possibility that piceatannol is also inhibiting PTKs other than Syk/Zap (or directly inhibiting Pyk2) cannot be ruled out. Thus, the precise role of Syk/Zap in CD28- and TCR-induced tyrosine phosphorylation of Pyk2 requires further investigation.

The PI 3-Kinase Inhibitor Wortmannin Inhibits TCR- but Not CD28-induced Tyrosine Phosphorylation of Pyk2—As the PI 3-kinase has been implicated in the signaling pathways of TCR and CD28, we examined the effect of the PI 3-kinase inhibitor wortmannin (71) on CD28- and TCR-induced tyrosine phosphorylation of Pyk2. Notably, wortmannin enhanced CD28- and TCR-induced tyrosine phosphorylation of several proteins (Fig. 6A). Similar enhancement by wortmannin of TCR-induced protein tyrosine phosphorylation has been previously reported (72). Treating the cells with wortmannin invariably decreased tyrosine phosphorylation of Pyk2 induced by aggregating TCR in a dose-dependent manner (Fig. 6B). In contrast, wortmannin had no apparent effect on CD28-induced tyrosine phosphorylation of Pyk2 (Fig. 6B). These data suggest that TCR and CD28 induce Pyk2 tyrosine phosphorylation by distinct pathways.

Depleting PMA-sensitive PKCs Does Not Block CD28- and
CD3-induced Pyk2 Tyrosine Phosphorylation—PKC is activated after CD28 and CD3 ligation (2, 3, 15, 16); thus, we examined whether receptor-mediated tyrosine phosphorylation of Pyk2 requires PKC. Jurkat cells were treated for 16 h with PMA (400 nM) to deplete Ca2+-dependent and Ca2+-independent PKC, and atypical PKCζ. As shown in Fig. 7A, PMA treatment of Jurkat T cells markedly reduced PKCs, as determined by blotting WCL with antibody to Ca2+-dependent PKCα and Ca2+-independent PKCδ. Atypical PKCζ was not decreased, as atypical PKCs are insensitive to PMA (73–75). Interestingly, the marked decrease in PMA-sensitive PKCs did not block CD28-induced Pyk2 tyrosine phosphorylation. These results suggest that receptor-mediated tyrosine phosphorylation of Pyk2 after CD28 ligation may be important for coupling CD28-initiated signals to downstream kinase pathways. Recent studies also link Pyk2 to the signaling pathways of the Rho family GTPases CDC42 and Rac1 (57). These GTPases are regulated by guanine nucleotide exchange factors such as Vav and by GTPases such as Ras and have been implicated in controlling the activation of JNK (37, 39, 40). Indeed, there is evidence implicating Pyk2 in pathways that regulate JNK activation. For example, overexpression of Pyk2 in human embryonic kidney cells activated JNK and induced the phosphorylation of the glutathione S-transferase-c-Jun fusion protein (57).

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REFERENCES
1. Aruffo, A., and Seed, B. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8573–8577
2. June, C. H., Ledbetter, J. A., Linsley, P. S., and Thompson, C. B. (1990) Immunol. Today 11, 211–216
3. Linsley, P. S., and Ledbetter, J. A. (1993) Annu. Rev. Immunol. 11, 191–212
4. Green, J. M., Noel, P. J., Sperling, A. L, Wahnas, T. L., Gray, G. S., Bluestone, J. A., and Thompson, C. B. (1994) Immunity 1, 501–508
5. Shahinian, A., Pfeffer, K., Lee, K. P., Kundig, T. M., Kishihara, K., Wakeham, A., Kawai, K., Ohashi, P. S., Thompson, C. B., and Mak, T. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8573–8577
Tyrosine Phosphorylation of Pyk2 by CD28 Ligation

Science 261, 609–612

6. June, C. H., Ledbetter, J. A., Gillespie, M. M., Lindsten, T., and Thompson, C. B. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 535–540

7. Bloemendaal, E., Van Der Heide, R., Smidts, P., and Van Der Vliet, A. (1995) J. Biol. Chem. 270, 2522–2527

8. Ledbetter, J. A., June, C. H., Groves, J. E., and Ruddle, C. E. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10981–10985

9. Bao, Y., Tan, D., Peng, Y., and Chen, C. (1994) Mol. Cell. Biol. 14, 109–116

10. June, C. H., Ledbetter, J. A., and Thompson, C. B. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 609–612

11. Nunes, J. A., Truneh, A., Olive, D., and Cantrell, D. A. (1996) J. Biol. Chem. 271, 1591–1598

12. Vandenbroeke, P., Freeman, G. J., Nadler, L. M., Fletcher, M. C., Kamsou, N., Turk, A. I., Ledbetter, J. A., Thompson, C. B., and June, C. H. (1993) J. Exp. Med. 178, 951–960

13. Klassen, S., Pages, F., Peyron, J. F., Cantrell, D. A., and Olive, D. (1996) Int. Immunol. 8, 179–185

14. Hamawy, M. M., Mergenhagen, S. E., and Siraganian, R. P. (1995) Cell Signalling 7, 535–544

15. Qian, D., and Weiss, A. (1997) Curr. Opin. Cell Biol. 9, 205–212

16. Cantrell, D. A. (1996) Annu. Rev. Immunol. 14, 259–274

17. Parsons, J. T. (1996) Curr. Opin. Cell Biol. 8, 146–152

18. Peri, K. G., and Veillette, A. (1994) Chem. Immunol. 59, 19–39

19. Wang, R. L., and Smeal, T. E. (1996) Immunity 5, 187–205

20. Chan, A. C., and Shaw, A. S. (1996) Curr. Opin. Cell Biol. 8, 394–401

21. Chan, A. C., Iwasawa, M., Turk, C. W., and Weiss, A. (1992) Cell 71, 649–662

22. August, A., Gibson, S. B., Waki, A., Matsuhashi, T., Mills, G. B., and DuPont, B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9347–9351

23. Lynch, K. L., Iwen, K., Clark, J. M., Phillips, C. A., and Trevillyan, J. M. (1992) J. Immunol. 149, 24–29

24. Hunter, D., and Bierer, B. E. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3260–3264

25. Laidlaw, J. A., and Linsley, P. S. (1992) Adv. Exp. Med. Biol. 323, 23–27

26. August, A., and Dupont, B. (1994) Biochem. Biophys. Res. Commun. 201, 1466–1473

27. Fazeli, P., Cai, Y. C., Raab, M., Dukworth, B., Cantley, L., Shoelson, S. E., Rudd, C. E. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2834–2838

28. August, A., and Dupont, B. (1994) Int. Immunol. 6, 769–774

29. Raab, M., Cai, Y. C., Bunnell, S. C., Heysen, S. B., Berg, L. J., and Rudd, C. E. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8881–8885

30. Liao, X. C., Fournier, S., Waki, A., Matsuhashi, T., Mills, G. B., and DuPont, B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2218–2218

31. Zou, F., Bhatia, R. K., and Vanger, D. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2937–2941

32. Pages, F., Lupien, M., Klassen, S., Baltierra, F., Dufort, J., Claret, B., Truneh, A., Ward, S. G., and Olive, D. (1996) J. Biol. Chem. 271, 9403–9409

33. Schneider, H., Cai, Y. C., Prasad, K. V., Shoelson, S. E., and Rudd, C. E. (1995) Eur. J. Immunol. 25, 1044–1050

34. Kim, H. H., Thurayyl, M., and Ruddle, C. E. (1996) J. Biol. Chem. 271, 296–301

35. Nunes, J. A., Collette, Y., Truneh, A., Olive, D., and Cantrell, D. A. (1994) J. Exp. Med. 180, 1067–1076

36. Sun, B., and Karin, M. (1996) Curr. Opin. Immunol. 8, 402–411

37. Kaga, S., Ragg, S., Rogers, K. A., and Ochi, A. (1998) J. Immunol. 160, 24–37

38. Vojtek, A. B., and Cooper, J. A. (1995) Cell 82, 527–529

39. Reif, K., and Cantrell, D. A. (1998) Immunity 8, 355–401

40. Sun, B., Casamento, E., Hibi, M., Kallunki, T., Karin, M., and Ben-Neriah, Y. (1994) Cell 77, 727–736

41. Hanks, S. K., Calab, M. B., Harper, M. C., and Patel, S. K. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8487–8491

42. Schaller, M. D., Borgman, C. A., Cobb, B. S., Vines, R. R., Reynolds, A. B., and Parsons, J. T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5192–5196

43. Le, V., Ragg, S., Roggen, B. A., and Ochi, A. (1998) J. Immunol. 160, 4182–4189

44. Felsch, J. S., Cachero, T. G., and Peralta, E. G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5505–5506