Protein Kinase C θ Cooperates with Vav1 to Induce JNK Activity in T-cells*

Andreas Möller, Oliver Dienz, Steffen P. Hehner, Wulf Dröge, and M. Lienhard Schmitz‡

From the German Cancer Research Center, Division of Immunochemistry (G0200), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

Received for publication, December 11, 2000, and in revised form, February 13, 2001
Published, JBC Papers in Press, March 23, 2001, DOI 10.1074/jbc.M011139200

* This work was supported by grants from the Land Baden-Württemberg, European Union (QLK3-2000-00463), Fonds der Chemischen Industrie, Deutsche Forschungsgemeinschaft (Schm 1417/3-1), and Deutsche Krebshilfe. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 49-6221-423725; Fax: 49-6221-423746; E-mail: L.Schmitz@DKFZ.de.

‡ The abbreviations used are: JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor-κB; IL, interleukin; PKC, protein kinase C; JNKK, JNK kinase; DN, dominant negative; EMSA, electrophoretic mobility shift assay; HA, hemagglutinin; GST, glutathione S-transferase.

Here we show that in human T-cell leukemia cells Vav1 and protein kinase C θ (PKCθ) synergize for the activation of c-Jun N-terminal kinase (JNK) but not p38 MAP kinase. Vav1 and PKCθ also cooperated to induce transcription of reporter genes controlled either by AP-1 binding sites or the CD28RE/AP composite element contained in the IL-2 promoter by stimulating the binding of transcription factors to these two elements. Dominant negative versions of Vav1 and PKCθ inhibited CD3/CD28-induced activation of JNK, revealing their relative importance for this activation pathway. Gel filtration experiments revealed the existence of constitutively associated Vav1/PKCθ heterodimers in extracts from unstimulated T-cells, whereas T-cell costimulation induced the recruitment of Vav1 into high molecular weight complexes. Several experimental approaches showed that Vav1 is located upstream from PKCθ in the control of the pathway leading to synergistic JNK activation. Vav1-derived signals lead to the activation of JNK by at least two different pathways. The major contribution of Vav1 for the activation of JNK relies on the PKCθ-mediated Ca2+-independent synergistic activation pathway, whereas JNK is also activated by a separate Ca2+-dependent signaling route.

Activation of the T-cell antigen receptor complex is not sufficient for T-lymphocyte activation and requires additional signals provided by the occupancy of costimulatory receptors such as CD28 (1). Full activation of T-cells by T-cell antigen receptor/CD28 costimulation initiates a series of intracellular signaling events. A hallmark of costimulated T-cells is the synergistic activation of JNK, NF-κB, and IL-2 expression (2). Receptor clustering leads to the activation of protein tyrosine kinases of the Src and Syk families, which phosphorylate numerous substrate proteins, thus leading to dynamic formation (3) or disruption (4) of multi-protein signaling complexes. These complexes and the T-cell antigen receptor itself associate with lipid rafts, which form a structural scaffold and promote further signaling events (5).

Activated protein tyrosine kinases phosphorylate multiple target proteins including phospholipase Cγ, which controls the phosphatidyl inositol lipid metabolism, thereby producing inositol triphosphate and diacylglycerols (6). Whereas inositol triphosphate results in a rapid and sustained calcium increase, diacylglycerol mediates activation of PKC family members (6, 7). Among those, the novel Ca2+-independent PKC isoform PKCθ is of special importance for T-cells, because it is rapidly recruited to the site of contact between T-cells and antigen-presenting cells (8). Another protein tyrosine kinase-induced signaling route is mediated by the Vav1 protein family member Vav1, which is exclusively expressed in hematopoietic cells (9). T-cell costimulation induces the membrane recruitment of Vav1 via indirect, adaptor protein SLP-76-mediated binding to the membrane protein LAT (linker for activation of T-cells) (10). Protein tyrosine kinase-induced phosphorylation and phosphatidylinositol-3,4,5-triphosphate binding of Vav1 activate its GDP/GTP exchange factor activity for the Rho family of GTPases such as Rac and Cdc42 (11, 12) and results in the stimulation of signaling pathways and alterations in cell shape and motility.

T-cell costimulation also leads to the activation of mitogen-activated protein kinase pathways. However, the synergistic activation appears to be unique for the mitogen-activated protein kinases JNK and p38 (13), because neither extracellular signal-regulated kinase nor transcription factor nuclear factor of activated T-cells require coreceptor-derived signals. JNK phosphorylates various transcription factors including ATF2, ELK-1, and components of the AP-1 heterodimer, namely JunB, JunD, and c-Jun (14). Because AP-1 contributes to the induced expression of numerous target genes including IL-2 and IL-4, the JNK pathway has been implicated in various functions including cell proliferation, effector T-cell function (15), T-cell activation (16), and the regulation of apoptosis (14). However, these functions are dependent on the inducing signal and the cell type (15, 16). JNK is activated by the dual specificity JNK kinases (JNKKs) MKK4/JNKK1/SEK1 and JNKK2/MKK7 (17). A variety of different kinases can activate the JNKKs, but the cell type and the nature of the JNK-inducing stimulus determines which of these kinases is operational (14).

Vav1 and PKCθ are constitutively associated in unstimulated T-cells (18), and both proteins synergistically activate transcription factor NF-κB, JNK activity, and the expression of IL-2, CD69, and IL-4 (4, 19, 20). In this study we have addressed the question whether Vav1 and PKCθ synergize for the activation of JNK for two reasons. 1) Gain-of-function approaches have revealed that both proteins contribute to the activation of JNK (10, 21–26) and cooperate with constitutively active calcineurin or Ca2+ signals to activate JNK. 2) We have previously seen that the Vav1/PKCθ module synergistically triggers binding of transcription factors to the P1 and PRE-I elements contained within the IL-4 promoter (19). Because both DNA elements are bound by AP-1 family members, we...
tested the effects of Vav1 and PKC\(\theta\) on the activation of JNK. A variety of experimental approaches revealed cooperative activation of JNK and AP-1-dependent gene expression by Vav1 and PKC\(\theta\).

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Transfections, and Stimulations**—Jurkat T leukemia cells expressing the large T antigen were grown in RPMI 1640 medium at 37 °C containing 10% (v/v) heat-inactivated fetal calf serum, 10 mM HEPES, 1% (v/v) penicillin/streptomycin, 2 mg/ml G418, and 2 mM glutamine (all from Life Technologies, Inc.). Cells were electroporated using a gene pulser (Bio-Rad) at 250 V/950 microfarads. In all transfections, the amount of total DNA (20 \(\mu\)g) was kept constant by the addition of empty expression vector. Stimulations were performed in a volume of 400 \(\mu\)l by adding agonistic cCD3/cCD28 antibodies as indicated. JNK was immunoprecipitated from cell lysates, and its activity was determined by immune complex kinase assays (\(KA\)) using recombinant GST-c-Jun(5–89) as substrate. An autoradiogram from a reducing SDS-gel shows phosphorylation of the recombinant substrate protein and a quantitative evaluation obtained by phosphorimaging. A sample of each lysate was analyzed by Western blotting (WB) for protein expression of PKC\(\theta\) (upper panel), JNK (middle panel), and Vav1 (lower panel). B, an expression vector for Flag-tagged \(p38\) (1 \(\mu\)g) and Vav1 and/or PKC\(\theta\) (5 \(\mu\)g, respectively) was transfected in Jurkat cells, which were either left untreated or costimulated for 30 min as shown. The tagged \(p38\) protein was immunoprecipitated and analyzed by Western blotting for \(p38\) expression and phosphorylation. Samples of whole cell lysates were immunoblotted for the expression levels of the various transfected proteins (lower panels).

**Antisera, Plasmids, and Reagents**—The following antibodies were obtained from the indicated suppliers: \(\alpha\)Phospho-\(p38\), New England Biolabs; \(\alpha\)Flag (M2), Sigma; \(\alpha\)Myc (9E10), Santa Cruz Biotechnology, Inc.; \(\alpha\)Vav1, Upstate Biotechnology; \(\alpha\)PKC\(\theta\), Transduction Laboratories; \(\alpha\)HA antibody (12CA5), Roche Molecular Biochemicals. The cCD3 (OKT3), cCD28 (9.3), and isotype-matched control antibodies were kindly provided by Dr. R. Breitkreutz. The luciferase constructs (AP-1\(_u\), AP-1\(_K\), 3\(x\)AP-1-Luc), 3\(x\)AP-1-Luc, and mutant derivatives thereof (27) and expression vectors for myc-tagged Vav1 wild type (27), Flag-tagged Vav1 wild type and DN Vav1 variants (10), Flag-tagged \(p38\) (28), PKC\(\theta\) A/E, PKC\(\theta\) K/R (19), MKK7 K/L (10), MEKK1 K/M (29), and MKK4 K/R (30) have been described.

**Electrophoretic Mobility Shift Assays (EMSAs) and Luciferase Determination**—EMSAs were performed using nuclear extracts essentially as described (19). Equal amounts of nuclear protein were tested for protein binding to oligonucleotides containing either an AP-1 binding site or a CD28RE/AP element. The coding strands of the oligonucleotides used were as follows: AP-1, 5’-CGTGTGATGACTCAGCCGGAA-3’; CD28RE/AP, 5’-TCTGTGGTTAAAAGAAAATTCGAAAGCAGCTCAGA-3’. The free and the oligonucleotide-bound proteins were separated by electrophoresis on a native 4% polyacrylamide gel. Following electrophoresis the gel was dried and exposed to an x-ray film (Amersham Hyperfilm).

Luciferase activity in cell extracts was measured in a luminometer (Duo Lumat LB 9507, Berthold) by automatically injecting 50 \(\mu\)l of assay buffer and measuring light emission for 10 s after injection according to the instructions of the manufacturer (Promega Inc.). To ensure comparable transfection efficiencies, results were normalized to \(\beta\)-galactosidase produced by a cotransfected Rous sarcoma virus-\(\beta\)galactosidase expression vector.

**Cell Extracts and Western Blotting**—Cells were washed with phosphate-buffered saline, and the pellets were resuspended on ice for 15 min in Nonidet P-40 lysis buffer (20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 mM NaF, 0.5 mM sodium vanadate, leupeptin (10 \(\mu\)g/ml), aprotinin (10 \(\mu\)g/ml), 1% (v/v) Nonidet P-40, and 10% (v/v) glycerol). Cell debris was pelleted upon centrifugation, and the supernatant was either directly analyzed by Western blotting or used for the determination of JNK activity as described below. After separation of cell extracts on reducing SDS-polyacrylamide gels, the
proteins were transferred onto a polyvinylidene difluoride membrane (Millipore) using a semi-dry blotting apparatus (Bio-Rad). The membrane was then incubated in a small volume of TBST buffer (25 mM Tris/HCl, pH 7.4, 137 mM NaCl, 0.7 mM CaCl$_2$, 20 mM MgCl$_2$, 0.1% (v/v) Tween 20) containing various dilutions of the primary antibodies. After extensively washing the membrane, the immunoreactive bands were visualized by enhanced chemiluminescence according to the instructions of the manufacturer (PerkinElmer Life Sciences). Western blots were quantitated using the Lumi-Imager™ from Roche Molecular Biochemicals.

**JNK Assays**—Two days posttransfection, cell lysates were prepared and precleared with protein A/G-Sepharose. The HA-tagged JNK proteins contained in the cell lysate were precipitated by the addition of 1 µg of αHA antibody and 25 µl of protein A/G-Sepharose. The precipitate was washed three times in lysis buffer and two times in kinase buffer (20 mM Hepes/KOH, pH 7.4, 25 mM β-glycerophosphate, 2 mM dithiothreitol, 20 mM MgCl$_2$). The kinase assay was performed in a final volume of 20 µl of kinase buffer containing 2 µg of glutathione S-transferase (GST)-c-Jun-(5–89), 20 µM ATP, and 5 µCi of [γ-32P]ATP for 20 min at 30 °C. The reaction was stopped by the addition of 5× SDS loading buffer, followed by reducing SDS-PAGE, gel fixation, and quantification of the results in a PhosphorImager.

**Gel Filtration**—The analysis of cellular multi-protein complexes was performed on a Superose 6 column (Amersham Pharmacia Biotech). Total cell extracts from $3 \times 10^8$ Jurkat cells contained in 75 mM octylglycoside lysis buffer (20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 5 mM dithiothreitol, leupeptin (10 µg/ml), aprotinin (10 µg/ml), 1% (v/v) octylglycoside, and 10% (v/v) glycerol) were analyzed at a flow rate of 0.5 ml/min, and fractions of 500 µl were collected. Aliquots from the respective fractions were analyzed by Western blotting for the occurrence of Vav1 and PKC$_{\alpha}$. The washed columns were calibrated with pre-made molecular weight standards (Amersham Pharmacia Biotech).

**RESULTS**

A possible synergism between Vav1 and PKC$_\alpha$ for the activation of JNK was tested by transfecting T-cell leukemia Jurkat cells with expression vectors for HA-tagged JNK together with vectors encoding constitutively active PKC$_\alpha$ (PKC$_\alpha$ A/E).
determine whether the synergy between Vav1 and PKC expression vectors for Vav1, PKC AP-1 binding to its cognate DNA, different combinations of whether this synergism is also apparent at the level of induced the cooperation only occurs for the activation of JNK. To test an additive, non-synergistic manner, thereby revealing that genetically stimulated upon coexpression of both proteins. Coexpression of the Vav1/PKC u experiments showed that Vav1 and PKC on protein binding to the CD28RE/AP element were investigated by EMSAs using the labeled CD28RE/AP element (Fig. 3B). Neither Vav1 nor PKC alone were able to induce DNA binding, but coexpression of both proteins caused DNA binding of transcription factors contained in the inducible DNA-protein complexes Ia and Ib. Formation of both complexes, which are known to contain predominantly c-Fos and c-Jun proteins (31), could be further triggered upon CD3/CD28 stimulation. In contrast to complex I, DNA-protein complexes II and III were already present in extracts from the untransfected, nonstimulated cells and showed no inducibility upon Vav1/PKC A/E coexpression and T-cell activation. The relatively moderate effects of Vav1/PKC on induced binding of proteins to the CD28RE/AP element can be attributed to the limited transfection efficiency of Jurkat cells. To investigate the relative importance of Vav1 and PKC for the CD3/CD28-mediated activation of JNK, the costimulation-induced activation of this mitogen-activated protein kinase was tested in the presence of DN versions of both signaling proteins. Coexpression of the kinase-deficient point mutant PKC K/R prevented JNK activation induced either by CD3/CD28 ligation or by treatment of cells with the pleiotropic PKC activator phorbol 12-myristate 13-acetate. Formation of both complexes, which are known to contain predominantly c-Fos and c-Jun proteins (31), could be further triggered upon CD3/CD28 stimulation. In contrast to complex I, DNA-protein complexes II and III were already present in extracts from the untransfected, nonstimulated cells and showed no inducibility upon Vav1/PKC A/E coexpression and T-cell activation. The relatively moderate effects of Vav1/PKC on induced binding of proteins to the CD28RE/AP element can be attributed to the limited transfection efficiency of Jurkat cells.

and/or wild type Vav1. The tagged JNK protein was immunoprecipitated, and its kinase activity was determined by immune complex kinase assays (Fig. 1A). JNK activation triggered by expression of Vav1 and PKC alone was synergistically stimulated upon coexpression of both proteins. Co-stimulation with agonistic αCD3/αCD28 antibodies further triggered JNK activity elicited by Vav1, PKC A/E, or both. To determine whether the synergy between Vav1 and PKC also occurs for the activation of p38, Jurkat T-cells were cotransfected with expression vectors for F-Flag-tagged p38 together with different combinations of vectors encoding Vav1 and PKC A/E. The tagged p38 protein was immunoprecipitated and analyzed for its activation (as seen by Thr-180/Tyr-182 phosphorylation) in Western blot experiments (Fig. 1B). These experiments showed that Vav1 and PKC activated p38 only in an additive, non-synergistic manner, thereby revealing that the cooperation only occurs for the activation of JNK. To test whether this synergism is also apparent at the level of induced AP-1 binding to its cognate DNA, different combinations of expression vectors for Vav1, PKC A/E, or the empty expression vector as a control were expressed in Jurkat cells. Analysis of AP-1 DNA binding activity by EMSAs showed that either Vav1 or PKC A/E alone induced DNA binding of AP-1, albeit to different extents. Coexpression of both proteins synergistically stimulated the DNA binding activity of AP-1 (Fig. 2B). The impact of Vav1/PKC expression on the activation of AP-1-dependent transcription was tested in reporter gene assays. An AP-1-dependent luciferase gene was transfected into Jurkat cells together with increasing amounts of Vav1 and/or PKC A/E expression vectors (Fig. 2B). The slight induction of AP1-dependent transcription mediated by Vav1 was strongly enhanced even by moderate amounts of coexpressed PKC A/E. These experiments revealed that the synergism also occurs at the level of DNA binding and gene expression.

JNK induces IL-2 promoter activity not only by targeting the AP-1 site but also via the CD28RE/AP composite element, which is bound by various proteins including members of the NF-κB/Rel and AP-1 families of transcription factors and so far only partially characterized proteins (27, 31). Because the CD28RE/AP element is absolutely required for the transmis-

![Fig. 4. Effects of DN forms of Vav1 and PKC on CD3/CD28-induced JNK activation.](image)
weight complexes of various sizes up to very large aggregates of more than 1 MDa (Fig. 5 B). In contrast, most of the PKC\(\theta\) protein coeluted with the 158-kDa marker protein, and only a minor fraction was found in larger complexes (Fig. 5 B).

The signaling pathways were characterized by monitoring the effect of coexpressed DN forms of protein kinases from the JNK activation pathway on the activation signals derived from Vav1, PKC\(\theta\), or both (Fig. 6 A). A kinase-dead form of MEKK1 affected Vav1-mediated AP-1 activity only moderately but significantly inhibited PKC\(\theta\)- and PKC\(\theta\)/Vav1-generated signals. A comparison between DN forms of the JNKKs MKK4 and MKK7 revealed that Vav1-derived activation signals were only moderately blocked by expression of DN forms of each of these kinases. In contrast, simultaneous expression of kinase-inactive forms of MKK4 and MKK7 completely prevented Vav1-mediated AP-1 activation, indicating that both kinases can mutually compensate the functions of each other. PKC\(\theta\) and Vav1/PKC\(\theta\)-derived signals were only partially impaired in the presence of DN MKK4 but efficiently blocked by MKK7 K/L, indicating the special importance of MKK7 for this pathway.

We also tested the impact of pathway-specific inhibitory compounds on Vav1- and/or PKC\(\theta\)-induced AP-1-dependent transcription (Fig. 6 B). Vav1-derived signaling, but not PKC\(\theta\)- and Vav1/PKC\(\theta\)-mediated AP-1 activation, was preferentially inhibited by cyclosporin A, a compound that blocks the Ca\(^{2+}\)-dependent activation of the phosphatase calcineurin. The PKC inhibitor bisindolylmaleimide blocked Vav1- and PKC\(\theta\)-mediated AP-1 activity, raising the possibility that Vav1 acts upstream from PKC\(\theta\). To address this question directly, Jurkat cells were transfected with various combinations of active and inactive variants of Vav1 and PKC\(\theta\) prior to stimulation with \(\alpha\text{CD3}/\alpha\text{CD28}\) antibodies and subsequent analysis of JNK activity. Vav1-induced JNK activation was efficiently prevented by kinase-dead PKC\(\theta\) K/R, but PKC\(\theta\) A/E-mediated JNK activation was not affected by DN Vav1 LLL/QIF (Fig. 7 A). To investigate whether directional signaling also occurs at the level of AP-1-dependent transcription, an analogous experimental approach was taken by monitoring AP-1-dependent luciferase activity. Similarly, Vav1-induced transcription of the AP-1-dependent luciferase gene was inhibited upon coexpression of PKC\(\theta\) K/R (Fig. 7 B) or the PKC inhibitor bisindolylmaleimide (data not shown). In contrast, gene activation induced by PKC\(\theta\) A/E was not affected by the DN Vav1 variant Vav1\(\Delta\)319–356. In summary, these data clearly indicate that PKC\(\theta\) acts downstream from Vav1 in a pathway leading to the activation of JNK.

**DISCUSSION**

Here we show that Vav1 and PKC\(\theta\) synergize for the induction of JNK. Because these two proteins also cooperate for the up-regulation of NF-\(\kappa\)B, the simultaneous activation of several pathways may be important for the efficient transcription of target genes, because many promoters (e.g. the IL-2 and IL-4 promoter) depend on the coordinated activation of several transcription factors. Interestingly, PKC\(\theta\) does not only synergize with Vav1 (this study) but also with calcineurin for the activation of JNK (22). The Vav1 protein cooperates with SLP-76 and Syk family kinases for the activation of IL-2 transcription and nuclear factor of activated T-cells activation, respectively (33, 34). One could speculate that the synergistic behavior of signaling proteins for the initiation of T-cell signaling pathways may be an important regulatory principle, especially early in infection when only low doses of antigen are present, but an efficient cellular response is required. In that respect it will be interesting to analyze signaling pathways and the immune response in Vav1/PKC\(\theta\) double knockout mice in future experiments.

In this study we show that at least the abundant fraction of
PKCθ and Vav1 proteins are present as heterodimers in unstimulated cells under the conditions used here. Given the importance of both proteins for the initiation of various signaling pathways (10, 32) and the necessity to keep activation pathways in nonstimulated T-cells silent (35, 36), this heterodimerization might serve the purpose of keeping both proteins in an inactive state. T-cell activation leads to the transient dissociation of Vav1 and PKCθ by an unknown mechanism (4) and the incorporation of Vav1 into temporally regulated and highly dynamic and semi-stable multi-protein signaling complexes (37). Under the conditions used here, only a minor fraction of the PKCθ protein is incorporated into high molecular weight complexes in stimulated T-cells. It will be interesting to investigate the composition, stability, and spatial and temporal regulation of these multi-protein complexes.

Our results indicate that Vav1 is located upstream from PKCθ in the JNK activation cascade. This finding is in good agreement with a previous study that demonstrated a Vav1-dependent membrane and cytoskeleton translocation of PKCθ (20). The same study shows that the effects of Vav1 on PKCθ are mediated by Vav1-induced actin polymerization and cytoskeletal reorganization. Biochemical and genetic evidence suggests that Vav1 exerts its function by at least two mechanisms. One pathway leads to the release of Ca²⁺ and the subsequent activation of the serine phosphatase calcineurin, which stimulates nuclear entry and transactivation of transcription factor nuclear factor of activated T-cells. This pathway is independent from the GDP/GTP exchange factor function of Vav1 (38) and cannot be inhibited by the regulatory protein Cbl-b (39). The second pathway is Ca²⁺-independent, can be inhibited by Cbl-b, and relies on the GDP/GTP exchange factor function of Vav1, thus leading to actin polymerization, cytoskeletal reorganization, and further processes (39). We favor a model where Vav1 and PKCθ activate JNK via overlapping and distinct pathways. The PKCθ-derived signals are Ca²⁺-independent and cannot be inhibited by calcineurin, because PKCθ is located downstream from it. Accordingly, a DN form of PKCθ inhibits JNK activation mediated by ionomycin,
which causes the release of intracellular Ca\(^{2+}\) (compare Fig. 4A). Similarly, the pathway shared by both signaling proteins and mediating the synergistic activation of JNK is Ca\(^{2+}\)/calcineurin-independent. In contrast, the Vav1-mediated JNK activation pathway contains a Ca\(^{2+}\)-dependent component that can be inhibited by cyclosporin A. This model would also explain earlier results that showed only moderate JNK activation by phorbol 12-myristate 13-acetate and ionomycin alone, whereas the simultaneous administration of both compounds strongly activated JNK activity (23).

This study suggests that Vav1-derived signals target MKK4 and MKK7. In contrast, PKC\(^\theta\) and Vav1/PKC\(^\theta\)-derived signals are transmitted preferentially via MKK7, which seems to be of special relevance for this pathway. The importance of MKK4 for the CD3/CD28-induced activation of JNK is not yet clear. One group describes a defective JNK activation in thymocytes from MKK4-deficient mice but normal JNK activation in peripheral T-cells (40). Another study demonstrates normal activation of JNK in response to CD3/CD28 stimulation in lymph node cell suspensions from MKK4\(^{-/-}\) mice (41). Further studies are required to resolve these differences, which may depend on the developmental stage of the lymphocytes and the cell types studied. In accordance with the predominant role of MKK7 described here, peripheral T-cells from mice lacking MKK7 show only low levels of JNK activity after CD3/CD28 stimulation (15).

The PKC\(^\theta\)-mediated signaling pathways are only incompletely understood. Furthermore, it is currently not known whether the competence of PKC\(^\theta\) to deliver activation signals is controlled by its intracellular localization, interaction with binding partners, or both. Because overexpression of the PKC\(^\theta\)-interacting protein, PKC-interacting cousin of thioredoxin, inhibits PKC\(^\theta\)-mediated activation of JNK and NF-\(\kappa\)B (42), this protein seems to be involved in the first signaling step prior to the separation of the two signaling pathways. Given the importance of PKC\(^\theta\) for the activation of NF-\(\kappa\)B and JNK, selective inhibition of this kinase might be a useful strategy to interfere with several activation pathways and thereby modulate T-cell costimulatory signals in inflammatory diseases.

Acknowledgments—We are grateful to Dr. Stephan Urban (Heidelberg) for help with gel filtration, Dr. Susanne Bacher and Ingrid Fryson for helpfull comments on the manuscript, Tarik Hamid for technical assistance, and colleagues who generously provided plasmids.

REFERENCES

1. Schwartz, R. H. (1997) Curr. Opin. Immunol. 9, 351–357
2. Jung, S., Yaron, A., Alkalay, I., Hatrutab, A., Avraham, A., and Ben-Neriah, Y. (1995) Ann. N. Y. Acad. Sci. 766, 245–252
3. Myung, P. S., Boerthe, N. J., and Koretzky, G. A. (2000) Curr. Opin. Immunol. 12, 256–260
4. Dienz, O., Hahn, S. P., Droge, W., and Schmitz, M. L. (2000) J. Biol. Chem. 275, 24547–24551
5. James, P. W., Ley, S. C., Magee, A. I., and Kabouridis, P. S. (2000) Semin. Immunol. 12, 23–34
6. Cantrell, D. (1996) Annu. Rev. Immunol. 14, 259–274
7. Bouchard, D., Kozieradzki, I., Ohashi, P. S., and Altman, A. (1998) J. Exp. Med. 188, 2999–2111
8. Hehner, S. P., Li-Weber, M., Giaiss, M., Droge, W., Krammer, P. H., and Schmitz, M. L. (2000) J. Immunol. 164, 3829–3836
9. Villalba, M., Coudronnieres, N., Deckert, M., Teixero, E., Mas, P., and Altman, A. (2000) Immunity 12, 151–160
10. Avraham, A., Jurgens, S., Sengers, W., and Ben-Neriah, Y. (1998) Eur. J. Immunol. 28, 2230–2239
11. Werlen, G., Jacinto, E., Xia, Y., and Karin, M. (1998) EMBO J. 17, 3101–3111
12. Gaffa-Habibi, N., Bauer, V., Vullinger, A., Baier-Bitterlich, G., Altman, A., Utermann, G., Uberall, F., and Baier, G. (1999) Eur. J. Immunol. 29, 132–142
13. Arudchandran, R., Brown, M. J., Peirce, M. J., Sung, J. S., Zhang, J., Siragianan, R. P., Blank, U., and Rivera, J. (2000) J. Exp. Med. 191, 47–60
14. Miranti, C. K., Leng, L., Maschberger, P., Brugge, J. S., and Shattil, S. J. (1998) Curr. Biol. 8, 1289–1299
15. Song, J. S., Hafeem-Smith, H., Arudchandran, R., Gomez, J. P., Mill, J. F., Tan, T. H., and Rivera, J. (1999) J. Immunol. 163, 802–810
16. Shapiro, V. S., Truitt, K. E., Imboden, J. B., and Weiss, A. (1997) Mol. Cell. Biol. 17, 4051–4058
17. Raingeaud, J., Gupta, S., Rogers, J. S., Dickman, M., Han, J., Ulevitch, R. J., and Davies, R. J. (1995) J. Biol. Chem. 270, 7420–7426
18. Coso, O. A., Chiariello, M., Yoo, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gotdikin, J. S. (1995) Cell 81, 1137–1146
19. Derijard, B., Raingeaud, J., Barrett, T., Wu, H. H., Han, J., Ulevitch, R. J., and Davies, R. J. (1995) Science 267, 682–685
20. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
21. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
22. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
23. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
24. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
25. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
26. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
27. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
28. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
29. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
30. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
31. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
32. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
33. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
34. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
35. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
36. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
37. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
38. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
39. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
40. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
41. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327
42. McGeire, K. L., and Iacobelli, M. (1997) J. Immunol. 159, 1319–1327