Inhibitory Role for Dual Specificity Phosphatase VHR in T Cell Antigen Receptor and CD28-induced Erk and Jnk Activation*

Received for publication, July 20, 2000, and in revised form, November 14, 2000
Published, JBC Papers in Press, November 20, 2000, DOI 10.1074/jbc.M006497200

Andres Alonso, Manju Saxena‡, Scott Williams, and Tomas Mustelin§

From the Laboratory of Signal Transduction, La Jolla Cancer Research Center, The Burnham Institute, La Jolla, California 92037

The 21-kDa dual specific protein phosphatase VH1-related (VHR) is one of the smallest known phosphatases, and its function has remained obscure. We report that this enzyme is expressed in lymphoid cells and is not induced by T cell antigen receptor like other dual specificity phosphatases. Introduction of exogenous VHR into Jurkat T cells caused a marked decrease in the transcriptional activation of a nuclear factor of activated T cells and an activator protein-1-driven reporter gene in response to ligation of T cell antigen receptors. The inhibition was dose-dependent and was similar at different doses of anti-receptor antibody. Catalytically inactive VHR mutants caused an increase in gene activation, suggesting a role for endogenous VHR in this response. In contrast, the activation of a nuclear factor κB-driven reporter was not affected. The inhibitory effects of VHR were also seen at the level of the mitogen-activated kinases Erk1, Erk2, Jnk1, Jnk2, and on reporter genes that directly depend on these kinases, namely Elk, c-Jun, and activator protein-1. In contrast, p38 kinase activation was not affected by VHR, and p38-assisted gene activation was less sensitive. Our results suggest that VHR is a negative regulator of the Erk and Jnk pathways in T cells and, therefore, may play a role in aspects of T lymphocyte physiology that depend on these kinases.

Members of the mitogen-activated protein kinase (MAP) kinase family are activated by a wide range of extracellular stimuli including growth and differentiation factors and cytokines as well as ultraviolet radiation, heat shock, and osmotic shock (reviewed in Refs. 1–3). Several distinct MAP kinase cascades have been identified in mammalian cells including the extracellular signal-regulated kinases Erk1 and Erk2, which preferentially transmit signals that regulate cell growth and differentiation, the c-Jun N-terminal kinases Jnk1 and Jnk2, the p38 kinases, which participate mainly in responses to stress, inflammation and apoptosis, and the 80-kDa Erk5, which may regulate cell proliferation (4). Activation of T lymphocytes by antigens is accompanied by activation of all of these pathways (5–8) although activation of the Jnks and p38s requires costimulation through CD28 (7), proinflammatory cytokines, or oxidative conditions that are often present at the site of inflammation and lymphocyte activation.

At the molecular level, MAP kinases are activated by a dual threonine and tyrosine phosphorylation within the motifs Thr-Glu-Tyr (Erk), Thr-Pro-Tyr (Jnk), or Thr-Gly-Tyr (p38) in their activation loops by a number of specific MAP kinase kinases (9). Conversely, inactivation of MAP kinases is achieved by dephosphorylation of either or both residues by protein Ser/Thr phosphatases, protein-tyrosine phosphatases, or dual specific protein phosphatases (DSPs) (reviewed in Refs. 10–12). In fact, the number of identified phosphatases that inactivate MAP kinases exceeds the number of known MAP kinase kinases, providing the first example of an important process in which the regulatory emphasis is more on the phosphatases than on the kinases (11).

Many members of the DSP group have assumed a role in MAP kinase inactivation, and most are inducible nuclear proteins that play important negative feedback roles in cellular signaling processes in response to environmental signals such as mitogenesis, apoptosis, differentiation, and secretion of cytokines (10–12). The first cloned member of this group was the VH1 protein from the vaccinia virus (13). A closely related enzyme was subsequently found in mammalian cells and termed VHR (VH1-related) (14). Although most DSPs identified to date seem to be specific for Erk1 and Erk2, and a few prefer Jnk or p38, the cellular target(s) for VHR have remained unclear. VHR also differs from other DSPs in being much smaller, only 21 kDa.

We report that VHR is expressed in all examined lymphoid and hematopoietic cell types and is constitutively expressed in T cells. We also present evidence that VHR counteracts the Erk and Jnk MAP kinases, but not p38, and all reporter genes that depend on Erk or Jnk. As a catalytically inactive VHR behaved as a “dominant-negative” in many experiments, we suggest that this may be the physiological function of VHR in T lymphocytes.

MATERIALS AND METHODS

Antibodies and Reagents—The anti-VHR mAb was from Transduction Laboratories (Los Angeles, CA). The 12CA5 anti-hemagglutinin mAb was from Roche Molecular Biochemicals. The 9E10 hybridoma producing the mAb that recognizes the c-Myc epitope tag and the OKT3 hybridoma that produces the anti-CD3ε mAb were from American Type Culture Collection (Manassas, VA). Both mAbs were used as ascites. The mAb against CD28 was from PharMingen (San Diego, CA). The
polyclonal anti-extracellular signal-regulated kinase 2 (Erk2) was from Santa Cruz Biotechnology Inc. A cDNA panel from hematopoietic and lymphoid organs was from CLONTECH (Palo Alto, CA).

Polymerase Chain Reactions and Plasmids—The cDNA for VHR was amplified from a Jurkat cell cDNA library using the polymerase chain reaction. The PCR product was sequenced and subcloned into the pEF/HA vector (15), which adds a hemagglutinin (HA) tag to the N terminus of the insert. The c-Myc-tagged Erk2 was in pEF-neo, the HA-tagged Erk1 (from G. Baier, University of Innsbruck, Austria) was in pEF-neo, HA-Jnk1 and HA-Jnk2 were in the pDNA3 vector, and p38-α cDNA (from J. D. Lee, Scripps Clinic, La Jolla, CA) was subcloned into the pEF-HA vector. GALA-Elk was from J. Tian and G. Hauser (Burnham Institute, La Jolla, CA) and GAL4-c-Jun and GAL4-luc were from T. Kawakami (La Jolla Institute for Allergy and Immunology, San Diego, CA).

Site-directed Mutagenesis—To generate a catalytically inactive mutant of VHR, the cDNA for Cys-124 was changed into a cDNA for serine in the pEF/HA-VHR plasmid using the Transformer™ site-directed mutagenesis kit as recommended by the manufacturer (CLONTECH). The substrate-trapping mutant of VHR (VHR-D92A) was generated using the Quick Change (Stratagene, San Diego, CA) site-directed mutagenesis kit as recommended by the manufacturer. Both mutations were verified by nucleotide sequencing.

Cells and Transfections—Jurkat T leukemia cells were kept at logarithmic growth in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM l-glutamine, 1 mM sodium pyruvate, nonessential amino acids, and 100 units/ml each of penicillin G and streptomycin. These cells were transiently transfected with a total of 5–10 μg of DNA by electroporation at 950 microfarads and 240 V. Empty vector was added to control samples to make constant the amount of DNA in each sample. Cells were used for experiments 24 h after transfection. Human bone marrow was purchased from AllCells LLC (Foster City, CA). Bone marrow leukocytes were obtained by hypotonic lysis of the erythrocytes in water for 1 min followed by washing of the cells in RPMI medium. Peripheral blood lymphocytes (~80% T cells) were obtained from venous blood from healthy donors (Red Cross Blood Bank, San Diego, CA) by Ficoll gradient centrifugation and by removal of monocytes by adherence to plastic at 37 °C for 2 h. The adherent cells were also recovered. Immunoprecipitation—Cells were lysed in 20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA containing 1% Nonidet P-40, 1 mM Na3VO4, 10 mM glycerol, 10 mM leupeptin, 100 μg/ml soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride and clarified by centrifugation at 15,000 rpm for 20 min. The clarified lysates were preabsorbed on protein G-Sepharose and then incubated with antibody for 2 h followed by protein G-Sepharose beads. Immune complexes were washed three times in lysis buffer, once in lysis buffer with 0.5M NaCl, again in lysis buffer, and either suspended in SDS sample buffer or used for in vitro kinase assays.

Immunoblotting—Proteins resolved by SDS-polyacrylamide gel electrophoresis were transferred electroblotically to nitrocellulose filters, which were immunoblotted as before (15–19) with optimal dilutions of mAbs followed by anti-mouse-Ig peroxidase. The blots were developed by the enhanced chemiluminescence technique (ECL kit, Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

Jnk Assays—These assays were performed as before (16). Briefly, 20 × 106 Jurkat T cells were transfected with either empty vector or 5 μg of HA-tagged Jnk1 or Jnk2 plasmid alone or plus 5 μg of VHR plasmid. Cells were harvested 2 days after electroporation, left untreated or stimulated with anti-CD3e mAb plus anti-CD28 (9.3) mAb and then incubated with antibody for 2 h followed by protein G-Sepharose beads. Immune complexes were washed three times in lysis buffer, once in lysis buffer with 0.5 mM NaCl, again in lysis buffer, and either suspended in SDS sample buffer or used for in vitro kinase assays.

Immunoblotting—Proteins resolved by SDS-polyacrylamide gel electrophoresis were transferred electroblotically to nitrocellulose filters, which were immunoblotted as before (15–19) with optimal dilutions of mAbs followed by anti-mouse-Ig peroxidase. The blots were developed by the enhanced chemiluminescence technique (ECL kit, Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

Jnk Assays—These assays were performed as before (16). Briefly, 20 × 106 Jurkat T cells were transfected with either empty vector or 5 μg of HA-tagged Jnk1 or Jnk2 plasmid alone or plus 5 μg of VHR plasmid. Cells were harvested 2 days after electroporation, left untreated or stimulated with anti-CD3e mAb plus anti-CD28 (9.3) mAb followed by the cross-linking with anti-mouse Ig for 20 min at 37 °C, or 10 μg/ml TNF-α, 1 μM ATP, 10 μg/ml of the activating anti-CD3e mAb OKT3 plus the anti-CD28 mAb for 0–48 h and 2 min. The samples were run on 10% SDS-polyacrylamide gels and transferred onto nitrocellulose filters, and the labeled proteins were visualized by autoradiography.

MAP Kinase Assays—These were done as before (17–19). Briefly, 200 × 106 Jurkat T cells were transfected with 5 μg of c-Myc-tagged Erk2 plasmid. Empty vector was added to control samples to make constant the amount of DNA in each sample. Cells were harvested 2 days after electroporation, divided into two samples/treatment, and either stimulated with OKT3 (5 μg/ml) for 5 min at 37 °C or left untreated. Cells were lysed as described above, and the Myc-tagged Erk2 was immunoprecipitated with 2 μg of the 9E10 anti-Myc mAb followed by 25 μl of protein G-Sepharose beads. The kinase reaction was performed for 30 min at 30 °C in 20 μl of kinase buffer containing 20 μM Tris/HCl, pH 7.5, 150 mM NaCl, 20 μM MgCl2, 10 μg/ml of myelin basic protein, 1 μM ATP, and 10 μCi of [γ-32P]ATP. The reactions were terminated by adding 20 μl of 2× SDS sample buffer and heating to 95 °C for 2 min. The samples were run on 12% SDS-polyacrylamide gels transferred onto nitrocellulose filters, and the labeled proteins were visualized by autoradiography.

Luciferase Assays—Luciferase assays were performed as described previously (16–19). Briefly, 20 × 106 cells were transfected with 2 μg of NFAF/ATP-1-luc (or other reporters) together with empty pEF/HA vector alone or plasmids. After stimulation for 6 h, the cells were lysed in 100 μl of 100 mM potassium phosphate, pH 7.8, 1 mM dithiothreitol, and 0.2% Triton X-100. The final assay contained 50 μl of lysis, 100 μl of ATP solution (10 mM ATP, 35 mM glycylglycine, pH 7.8, and 20 μM MgCl2), plus 100 μl of luciferin reagent (0.27 μM coenzyme A, 0.47 μg/ml luciferin, 35 mM glycylglycine, pH 7.8, and 20 μM MgCl2). The activity was measured in an automatic luminometer (Monolight 2010, Analytical Luminescence Laboratory, Ann Arbor, MI). The activity of a co-transfected β-galactosidase was measured and used to normalize the luciferase activity for transfection efficiency.

RESULTS

Expression of VHR in Hematopoietic and Lymphoid Cells—To survey normal lymphoid cell types and organs for VHR expression, we designed an oligonucleotide primer pair complementary to the ends of the open reading frame to amplify VHR mRNA-derived sequences by the PCR from a panel of cDNA libraries (CLONTECH). A fragment of the expected size (558 base pairs) was readily obtained from bone marrow, fetal liver, lymph node, peripheral blood lymphocytes, spleen, thymus, tonsil, and the T leukemia cell line Jurkat (Fig. 1A). The expression was lowest in the thymus and highest in Jurkat. To verify that the amplification products were derived from VHR, we cloned and sequenced the fragment. The obtained sequence was 100% identical to the published sequence of VHR (14) (data not shown).

To confirm the presence of VHR protein in hematopoietic cells, cell lysate samples containing 70 μg of protein from isolated blood T cells, monocytes, bone marrow leukocytes, or Jurkat T cells were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and immunoblotted with the anti-VHR mAb. An ~21-kDa band of very similar intensity was seen in these cells (Fig. 1A, right panels).

Generation of a HA-tagged VHR Expression Plasmid—Next we subcloned the sequenced open reading frame of VHR into the pEF/HA vector, which adds an N-terminal HA tag to the insert. When the plasmid was transfected by electroporation into Jurkat T cells, a 23-kDa protein appeared as expected (the HA tag adds ~2 kDa). This protein was immunoprecipitated by both an anti-HA mAb and an anti-VHR mAb and was immunoblotted by both mAbs (Figs. 1B and 2). In contrast, an endogenous protein of 21 kDa was immunoprecipitated and immunoblotted with only the anti-VHR mAb (Fig. 1B and C).

Cell Activation Does Not Induce VHR Expression—Because most DSPs are inducible genes encoded by immediate early genes, we wanted to determine whether this was also true for VHR. Jurkat T cells were treated with 5 μg/ml of the activating anti-CD3e mAb OKT3 plus the anti-CD28 mAb for 0–48 h and

T Cell Antigen Receptor Inactivates VHR
then immunoblotted with the anti-VHR mAb. The amount of VHR immunoreactivity remained completely unchanged during these experiments (Fig. 1C). The same experiment was also performed with normal blood T cells with the same result (Fig. 1D). Thus, VHR is constitutively expressed in T cells prior to receptor stimulation and is not induced.

Active VHR Inhibits Anti-CD3 Plus Anti-CD28-induced Activation of a Reporter Gene Taken from the Interleukin-2 Gene—As a rapid screening assay for a possible role of VHR in T cell activation, we coexpressed VHR with a sensitive luciferase reporter gene, in which luciferase transcription is under the control of a tandem NFAT/AP-1 element taken from the interleukin-2 gene promoter. This reporter responds to T cell antigen receptor ligation with anti-CD3 mAbs alone or together with coligation of the CD28 costimulatory molecule. When these experiments were first performed with different amounts of VHR plasmid, we observed that VHR inhibited the response in a dose-dependent manner with a sharp drop in gene activation between 2 and 5 μg of plasmid (Fig. 2A). The expression of VHR protein correlated with the DNA dose (inset). A titration of the amount of anti-CD3 e showed that VHR did not shift the anti-CD3 e dose-response (e.g. by desensitizing the receptor) but inhibited it to an equal degree at all doses (Fig. 2B).

In contrast to the effect of catalytically active VHR, expres-
sion of the two catalytically inactive VHR mutants, C124S and D92A, had the opposite effect, namely a substantial increase in gene activation in response to anti-CD3e or anti-CD3ε plus anti-CD28 (Fig. 2C). This dominant-negative effect may result from a displacement of endogenous, active VHR. If so, our results support the notion that endogenous VHR suppresses reporter gene activation. Catalytically active VHR also inhibited reporter gene activation induced by phorbol ester and ionomycin, whereas the inactive C124S mutant augmented it (Fig. 2D). In support of a role of VHR in the signaling pathways that up-regulate NFAT/AP-1-driven transcription, we found that coexpression of VHR with a luciferase reporter under the control of a nuclear factor κB element had a small stimulatory effect on its activation (Fig. 2E). Because nuclear factor κB induction, in contrast to NFAT/AP-1, was not inhibited in a dose-dependent manner, we conclude that VHR does not inhibit this pathway even when expressed at higher levels. The small stimulatory effect is of questionable significance but could be secondary to inhibition of other pathways.

Active VHR Inhibits Anti-CD3 Plus Anti-CD28-induced Activation of Jnk—To further understand the effect of VHR on reporter gene activation and to address the hypothesis that VHR acts on one or several members of the MAP kinase family, we measured the activation of these kinases one by one. First, VHR was cotransfected with an epitope-tagged Jnk2 in Jurkat T cells. Two days after transfection, the cells were treated with anti-CD3ε plus anti-CD28 on ice for 15 min, washed twice with cold RPMI, and then incubated at 37 °C for 20 min. Both treatments activated Jnk2, and both were inhibited by active VHR (Fig. 3, lanes 9–24). Activation of Jnk2 by heat shock was augmented ~2-fold by the catalytically inactive VHR mutant (39,203 cpm in lane 24 versus 17,333 cpm in lane 20 incorporated into the substrate), but the very strong activation of Jnk2 by ultraviolet light was partly reduced by this mutant. This finding was consistently seen in several independent experiments and may be due to binding to Jnk2 resulting in some steric hindrance of the kinase. The amount of Jnk2 was found to be similar in all the samples (lower panels), and VHR and VHR-C124S were equally expressed in lanes 13–16 and 21–24 (lower panels). These results were obtained in two independent experiments.

Inhibition of Erk Activation by Active VHR—Having established that Jnk is sensitive to VHR, we tested Erk activation in similar experiments. A Myc-tagged Erk2 was coexpressed with VHR or inactive mutant VHR, and the cells were stimulated with the anti-CD3ε mAb for 5 min (anti-CD28 is not required). After cell lysis, Erk2 was immunoprecipitated with the 9E10 anti-Myc mAb and subjected to an in vitro kinase reaction with myelin basic protein as a substrate. These experiments revealed that Erk activation was reduced by active VHR but not by the inactive mutant (Fig. 4A). However, in most experiments, the inhibition was not as marked as the inhibition observed for Jnk. The amount of Erk was equal in all samples (Fig. 4A, middle panel), and VHR was well expressed (Fig. 4A, bottom panel). This result was obtained in several additional experiments.

VHR Has No Effects on Receptor- or UV-induced Activation of p38 Kinase—p38 kinase is also activated in T cells in response to receptor signaling and ultraviolet irradiation. However, coexpression of active or inactive VHR had no effect on these responses despite being well expressed (Fig. 4B). These results have been obtained in several independent experiments and suggest that VHR has specificity toward Jnk and Erk but not p38.

Effects of VHR on Downstream Targets for MAP Kinases—To further investigate the negative regulation of Jnk and Erk by VHR, we decided to utilize a number of downstream targets of these kinases. In intact cells, many of these targets are also affected by p38, but the relative importance of the three principal MAP kinases varies considerably from target to target. First we utilized a luciferase reporter gene under the control of the Elk protein, which is phosphorylated and activated mostly by Erk. To increase or decrease its dependence on Erk, we coexpressed Erk, Jnk, or p38 with the reporter. These experi-
ments showed that the reporter was sensitive to VHR and even more sensitive when the level of Erk was increased (Fig. 5A).

Coexpression of p38 caused a smaller augmentation of reporter gene activation, and the inhibition was less striking, as would be expected if p38 were more involved relative to Erk in these cells. The effects of Jnk were intermediate.

The activation of the c-Jun protein is principally mediated by Jnk and can be measured using a c-Jun-GAL4 fusion protein, which controls the expression of a cotransfected GAL4-sensitive luciferase (20). This reporter was activated 2-fold by stimulation of the T cells with anti-CD3 e plus anti-CD28 but was much augmented by coexpression of Jnk1 or Jnk2 (Fig. 5B). In both cases, a coexpressed VHR caused a strong inhibition of the response.

Finally we used a luciferase reporter gene driven by a single AP-1 site (without an adjacent NFAT element) that binds the dimeric Jun/Fos complex referred to as AP-1. This reporter was strongly induced when either Jnk or Erk was coexpressed, and in both cases VHR caused a marked inhibition of the response. In contrast to the Elk reporter, the AP-1 reporter was somewhat better responsive to Jnk and more inhibited by VHR in the presence of Jnk. Nevertheless, all these results show that both Erk and Jnk are similarly sensitive to inhibition by VHR, whereas p38 is much less so, if at all.

FIG. 4. VHR inhibits Erk activation but not p38 kinase activation. A, in vitro kinase assay with myelin basic protein (MBP) as a substrate of Myc-tagged Erk2 immunoprecipitated from Jurkat T cells transfected with the indicated plasmids and treated with medium (odd lane numbers) or anti-CD3e mAb (even lane numbers) for 5 min. Equal amounts of Erk in the assay were verified by anti-Erk immunoblotting of the same filter (middle panel) and the expression of VHR by anti-HA immunoblotting of lysates from the same transfectants (lower panel). B, in vitro kinase assay (upper panel) with GST-ATF2 as a substrate of HA-tagged p38 immunoprecipitated from Jurkat T cells transfected with the indicated plasmids and treated with medium alone (odd lanes), UV irradiation (lanes 2, 4, 6, and 8), or anti-CD3e (lanes 10, 12, 14, and 16) for 5 min. Equal expression was verified by anti-HA immunoblotting of lysates from the same transfectants (lower panel).

FIG. 5. Inhibition of downstream targets for MAP kinases by VHR. A, activation of a luciferase reporter gene driven by Elk (Elk-GAL4-luc). The data represent the mean ± S.D. from triplicate determinations. The right-hand panels represent an anti-Erk2 blot (upper panel), a longer exposure of the anti-HA blot to show Jnk (middle panel), and a shorter exposure of the anti-HA blot to show p38 and VHR (lower panel) of the lysates of the transfectants. Note that endogenous Erk with a slightly lower Mr is visible in all lanes in the anti-Erk2 blot. B, activation of a c-Jun-induced luciferase reporter gene (c-Jun-GAL4 luc). The data represent the mean ± S.D. from triplicate determinations. The right-hand panel is an anti-HA blot to show Jnk and VHR. C, activation of a luciferase reporter gene driven by AP-1 (AP-1-luc). The data represent the mean ± S.D. from triplicate determinations. The right-hand panel is an anti-HA blot to show Jnk1, Erk1, and VHR.

DISCUSSION

The 185-amino acid VHR was the first human DSP to be cloned (14); the enzyme has been crystallized, and its three-dimensional structure has been solved (21). The structure revealed that the catalytic center of VHR is composed of the same structural elements as in the classical protein-tyrosine phosphatases like PTP1B (22) including the cysteine (Cys-124) in the bottom of the catalytic cleft. Mutagenesis experiments and kinetic analyses have confirmed that Cys-124 is essential for catalysis (23, 24) and that Asp-92 participates in substrate dephosphorylation by acting as proton donor for the reaction (25). The dual specificity of VHR toward both phosphotyrosine and phosphoserine/phosphothreonine in protein substrates is explained by its catalytic pocket being less deep than in other protein-tyrosine phosphatases. This allows all three phosphoamino acid residues to reach the catalytic machinery (21). However, dephosphorylation of phosphotyrosine is much preferred over phosphothreonine and occurs much more rapidly (26).

Despite the detailed insights into its atomic structure and catalytic mechanism, the physiological function of VHR has
remained obscure. It differs from the group of MAP kinase-specific DSPs in that it lacks noncatalytic regulatory or target- ing regions. For this reason, the enzyme is also considerably smaller than other DSPs, only 21 kDa. Nevertheless, a recent paper (27) reported that VHR can dephosphorylate Erk1 and Erk2 and suggested that these two kinases are physiological targets for VHR. Our findings agree with this report, but we also find that Jnk is an equally preferred target for VHR in intact T cells. Like Todd et al. (27), we find that p38 kinase is not affected. It remains to be determined what gives VHR this specificity and how the enzyme is targeted and regulated.

In T cells, the Erk kinases are activated very rapidly with peak activities observed within minutes followed by a gradual decline beginning within 5 min and a return to basal or near basal levels at 10–30 min. The inducible DSPs, primarily the Pac-1 protein in lymphocytes (28), are not present at these time points but appear in the nucleus some 30–60 min after cell stimulation. In this location, they can dephosphorylate any remaining activated MAP kinases and terminate the response. In fibroblasts lacking MKP-1 (29), also encoded by an immediately early gene (30), the activation of MAP kinase also proceeds normally. In contrast, when MKP-1 is expressed in cells under a noninducible promoter, it strongly blocks MAP kinase activation by active Ras (31) or by extracellular stimuli (30, 32, 33). At the early time points, however, MAP kinase activation must be counteracted by phosphatases that are present in resting cells such as hematopoietic protein-tyrosine phosphatases (17, 18) and VHR. At present, no other MAP kinase-specific phosphatases are known to be constitutively expressed in lymphocytes.

In its role as a negative regulator of Erk, VHR may be competing with a number of other phosphatases (reviewed in Ref. 11). However, as a Jnk regulator VHR is accompanied only by VHS (M3/6 in the mouse (34)) and perhaps by MKP-2, which dephosphorylates both Erk and Jnk with comparable efficiency at least in vitro (35). Both of these enzymes are inducible nuclear enzymes that may not be present in T cells at least during the first 30–60 min after receptor stimulation. Thus, VHR may be of particular importance in this cell type as a negative regulator of Jnk1 and Jnk2, which are important signal transducers in lymphocytes as well as other cell types (36, 37).

Acknowledgments—We are grateful to Drs. Gottfried Baier, J. D. Lee, Jianmin Tian, Greg Hauser, and Toshiaki Kawakami for the kind gift of reagents.

REFERENCES

1. Kyriakis, J. M., and Avruch, J. (1996) J. Biol. Chem. 271, 24313–24316
2. Robinson, M. J., and Cobb, M. H. (1997) Curr. Opin. Cell Biol. 9, 180–186
3. Schaeffer, H. J., and Weber, M. J. (1999) Mol. Cell. Biol. 19, 4235–4241
4. Kato, Y., Krivchenko, V. V., Tapping, R. I., Han, J., Ulevitch, R. J., and Lee, J. D. (1997) EMBO J. 16, 7054–7066
5. Nel, A. E., Hanekom, C., Eedder, A., Williams, K., Pollack, S., Katz, R., and Landreth, G. (1990) J. Immunol. 144, 2683–2689
6. Whitehurst, C. E., and Geggert, T. D. (1996) J. Immunol. 156, 1020–1029
7. Su, B., Jacinto, E., Hibi, M., Kallunki, T., Karin, M., and Ben-Neriah, Y. (1994) Cell 77, 727–736
8. Salmin, R. A., Foltz, I. N., Young, P. R., and Schrader, J. W. (1997) J. Immunol. 159, 5309–5317
9. Canagarajah, B. J., Khoklatchev, A., Cobb, M. H., and Goldsmith, E. J. (1997) Mol. Cell. Biol. 17, 47205–47208
10. Keyse, S. M. (2000) Curr. Opin. Cell Biol. 12, 186–192
11. Saxena, M., and Mustelin, T. (2000) Semin. Immunol. 12, 387–396
12. Camps, M., Nichols, A., and Arkingall, S. (1999) FASEB J. 14, 6–16
13. Guan, K. L., Bryoles, S. S., and Dixon, J. E. (1991) Nature 350, 359–362
14. Ishibashi, T., Bortto, D. P., Chan, A. K., and Aaronson, S. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 12170–12174
15. von Willebrand, M., Jascur, T., Bounez-Berdar, N., Yano, H., Altman, A., Matsuda, Y., and Mustelin, T. (1996) Eur. J. Biochem. 235, 828–835
16. Gjorloff-Wingren, A., Saxena, M., Williams, S., Hammi, D., and Mustelin, T. (1999) Eur. J. Immunol. 29, 3845–3854
17. Saxena, M., Williams, S., Brockdorff, J., Gilman, J., and Mustelin, T. (1999) J. Biol. Chem. 274, 11693–11700
18. Saxena, M., Williams, S., Tasken, K., and Mustelin, T. (1999) Nat. Cell Biol. 1, 305–311
19. Jascur, T., Gilman, J., and Mustelin, T. (1997) J. Biol. Chem. 272, 14483–14489
20. Hata, D., Kitaura, J., Hartman, S. E., Kawakami, Y., Yokota, T., and Kawakami, T. (1998) J. Biol. Chem. 273, 10979–10987
21. Yuvaniyama, J., Denu, J. M., Dixon, J. E., and Saper, M. A. (1996) Science 272, 1328–1331
22. Barford, D., Flint, A. J., and Tonks, N. K. (1994) Science 263, 1397–1404
23. Zhou, G., Denu, J. M., Wu, L., and Dixon, J. E. (1994) J. Biol. Chem. 269, 28084–28089
24. Denu, J. M., and Dixon, J. E. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5910–5914
25. Denu, J. M., Zhou, G., Guo, Y., and Dixon, J. E. (1995) Biochemistry 34, 3640–3645
26. Denu, J. M., Zhou, G., Wu, L., Zhao, R., Yuvaniyama J., Saper, M. A., and Dixon, J. E. (1995) J. Biol. Chem. 270, 3796–3803
27. Todd, J., Tannier, R., and Dent, J. (1999) J. Biol. Chem. 274, 12271–12280
28. Rohan, P. J., Davis, P., Mokkuluk, C. A., Kearns, M., Krutzsch, H., Siewbenlist, U., and Kelly, K. (1993) Science 259, 1763–1766
29. Dorfman, K., Carassco, D., Gruda, M., Ryan, C., Lira, S. A., and Bravo, R. (1996) Oncogene 13, 295–301
30. Charles, C. H., Sun, H., Lau, L. F., and Tonks, N. K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5292–5296
31. Sun, H., Tonks, N. K., and Bar-Sagi, D. (1994) Science 266, 265–288
32. Sun, H., Charles, C. H., Lau, L. F., and Tonks, N. K. (1994) Cell 75, 487–493
33. Noguchi, T., Metz, R., Chen, L., Mattei, M. G., Carassco, D., and Bravo, R. (1993) Mol. Cell. Biol. 13, 5185–5205
34. Mutia, M., Theodosiou, A., Rodrigues, N., Bouchet, U., Camps, M., Gillieron, C., Davies, K., Ashworth, A., and Arkingall, S. (1996) J. Biol. Chem. 271, 27205–27209
35. King, A. G., Ozanne, B. W., Snythe, C., and Ashworth, A. (1995) Oncogene 11, 2553–2563
36. Sabapathy, K., Hu, Y., Kallunki, T., Schreiber, M., David, J. P., Jochum, W., Wagner, E. F., and Karin, M. (1999) Curr. Biol. 9, 116–125
37. Chu, W. M., Ostertag, D., Li, Z. W., Chang, L., Chen, Y., Hu, Y., Williams, B., Perrault, J., and Karin, M. (1999) Immunity 11, 721–731