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The Raf-1/Mitogen-Activated Protein Kinase Kinase-1/Extracellular Signal-Regulated-2 Signaling Pathway as Prerequisite for Interleukin-2 Gene Transcription in Lectin-Stimulated Human Primary T Lymphocytes

*Virginie Lafont, Bruno Rouot and Jean Favero**

INSERM U431, Microbiologie et Pathologie Cellulaire Infectieuse, Universite de Montpellier II, Place Eugene Bataillon, cc 100, 34095 Montpellier cedex 05, France

ABSTRACT. It has been shown that stimulation of lymphoid cells causes the activation of the extracellular signal-regulated-2 (ERK-2) which activates nuclear factor of activated T cells (NF-AT), a transcription factor involved in the regulation of interleukin-2 (IL2) gene transcription. ERK-2 is activated via a kinase cascade initiated by activation of the G protein p21Ras followed by phosphorylation and activation of Raf-1 and mitogen-activated protein kinase kinase-1 (MEK-1). Activation of this pathway has been described primarily in human T cell lines; however, using primary T lymphocytes from transgenic mice, a recent study has shown that a blockade of this cascade did not perturb lymphocyte stimulation and proliferation. In the present paper, we studied in human primary T cells the possible involvement of the Raf-1/MEK-1/ERK-2 pathway upon stimulation by jacalin, a mitogenic lectin which specifically stimulates $CD4^+$ lymphocytes. We show here that the mitogen-activated protein (MAP) kinase pathway was stimulated in human purified lymphocytes upon activation with jacalin. Moreover, activation of this pathway appeared to be essential, since its blockade by a specific inhibitor of the MEK-1 kinase abolished IL2 gene transcription; in contrast, in T cells stimulated with phytohemagglutinin M(PHA), another potent T cell mitogenic lectin, blockade of MEK-1 reduced but did not totally inhibit either ERK-2 phosphorylation or IL2 mRNA expression. This shows, as already suggested, that another pathway in addition to the Raf-1/MEK-1/ERK-2 kinase cascade could be triggered in T cell activation. Jacalin stimulation therefore appeared to be a good model for the specific activation of the MAP kinase pathway in human primary T lymphocytes, which would allow the characterisation of drugs specifically targeted to this particular pathway. BIOCHEM PHARMACOL **55**;3:319–324, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. MAP-kinase; ERK-2; jacalin; T lymphocyte; tyrosine phosphorylation

Stimulation of the T cell antigen receptor complex causes a series of intracellular biochemical events that regulate the production of several cytokines, including interleukin-2 (IL2)†, and that promote T cell proliferation. Regulation of IL2 gene transcription is dependent on the coordinate action of multiple transcription factors including nuclear factor of activated T cells (NF-AT), activated protein-1 (AP-1), nuclear factor κB (NF-κB) and octamer protein-1 (Oct-1) [1, 2]. Activation of these transcription factors is dependent on both the stimulation of the calcium/calcineurin pathway and p21Ras-mediated signaling pathways [3, 4]. Accumulating evidence shows that these pathways are initiated by $p56^{lck}$ and $p59^{fyn}$ [5, 6], which phosphorylate tyrosine residues on the ε and ζ chains of the CD3/TcR complex, allowing the binding and activation of the syk-family kinase ZAP-70 [3, 7]. The coupling of these protein tyrosine kinases (PTK) to the TcR controls the activation of the low molecular weight G protein p21Ras [5, 8]. At the plasma membrane, active guanosine triphosphate (GTP)-bound Ras directly binds and promotes activation of the protein kinase Raf-1, which phosphorylates and activates a MAPK Kinase (MAPKK, MEK), which in turn phosphorylates and activates the MAP kinase ERK-1/2 [9, 10]. However, if many results using human T cell lines have provided some evidence that IL2 gene transcription is promoted from Ras and Raf-1 relayed through MEK-1 and ERK-2 [11, 12], a recent study using T cells from transgenic mice that express substituted $MEK\text{-}1_{A97}$ [13] (alanin for lysine substitution at position 97) and results from our laboratory (unpublished data) have provided evidence that the MAP kinase cascade is not an obligatory and exclusive pathway in TcR/CD3-triggered T cell stimulation.

We have recently shown that jacalin, a lectin purified

* Corresponding author: Dr. Jean Favero, INSERM U431, Microbiologie et Pathologie Cellulaire Infectieuse, Université de Montpellier II, Place Eugène Bataillon, cc 100, 34095 Montpellier cedex 05, France. Tel. +(33) 467 14 42 44; FAX +(33) 467 14 33 38; E-mail: favero@univ-montp2.fr.

† *Abbreviations:* PTK, protein tyrosine kinase; IL2, interleukin-2; PHA, phytohemagglutinin M; PMA, phorbol 12-myristate 13-acetate; MBP, myelin basic protein. MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; MEK, MAP kinase kinase; NF-AT, nuclear factor of activated T cells.

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from jackfruit seeds which specifically stimulates $CD4^+$ lymphocytes [14, 15] and inhibits *in vitro* HIV-1 infection [16], is able to trigger cell signaling through the CD4 antigen [17, 18] via activation of the CD4-associated protein tyrosine kinase p56lck.* We showed using $CD4^+$ Jurkat cells lacking the TcR/CD3 complex that jacalin triggers via CD4, intracellular calcium increase through an uncommon mechanism involving calcium influx without stimulation of the phosphoinositide-phospholipase C γ1 pathway and without calcium release from intracellular stores [18, 19]. We therefore questionned in the present paper if IL2-mRNA expression specifically induced in peripheral blood $CD4^+$ lymphocytes by jacalin involves the activation of the Raf-1/MEK-1/ERK-2 kinase cascade.

MATERIALS AND METHODS

Chemicals and Reagents

Jacalin was purchased from Lectinola and phytohemagglutinin M (PHA) from Difco. Phorbol 12-myristate 13-acetate (PMA) and myelin basic protein (MBP) were from Sigma. Mouse anti-phosphotyrosine mAb (4G10) was from Upstate Biotechnology Inc., rabbit anti-ERK-2 Ab and rabbit anti-Raf-1 Ab were from Santa Cruz Biotechnology. Horseradish peroxidase-conjugated rabbit anti-mouse and donkey anti-rabbit Ab were from Amersham.

Cell Preparation and Culture

Mononuclear cells were isolated on ficoll-hypaque from peripheral blood from healthy donors. Monocytes were purified by plastic adherence. $CD4^+$ T cells were purified by positive immunoselection using magnetic beads coated with anti-CD4 mAb (Dynal International) according to the manufacturer's instructions. The cells analyzed by flow cytometry (FACScan, Becton Dickinson) were >99% pure. The cells (10^6/mL) were cultured in RPMI 1640 supplemented with 10% fetal calf serum and gentamycin at 37° in a 5% CO_2 humidified atmosphere.

Analysis of IL2 mRNA Expression by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

$CD4^+$ T cells supplemented with 20% autologous monocytes were cultured for an optimal period of 6 hr at 37° in the presence of jacalin (50 μg/mL) or PHA (10 μL/mL). Total RNA was isolated with Trizol® (Life Technologies SARL) as described by the manufacturer. The reverse transcription reaction was performed at 42° for 90 min on 5 μg total RNA, using the Murine Moloney-Leukemia-Virus Reverse Transcriptase (M-MLV-RT, Life Technologies), oligo dT (Life Technologies) in the presence of 1 μCi ^{32}P-dCTP (ICN, Orsay, France) to allow a quantification of synthesized cDNA. 0.1 ng of each cDNA was amplified using 2.5 units Gold Star Polymerase (Eurogentec) and 1 μM specific primers (Eurogentec), IL2: 5′-GTC-ACA-AAC-AGT-GCA-CCT-AC-3′ (sense); 5′-ATG-GTT-GCT-GTC-TCA-TCA-GC-3′ (antisense); β_2 microglobulin: 5′-CCA-GCA-GAG-AAT-GGA-AAG-TC-3′ (sense), 5′-GAT-GCT-GCT-TAC-ATG-TCT-CG-3′ (antisense). The number of cycles was fixed at 30 and amplification of β_2-microglobulin was used as a control.

Analysis of Tyrosine Phosphorylation

Tyrosine phosphorylation analysis was performed according to Hivroz *et al.* [20]. Briefly, 5.10^6 purified $CD4^+$ T cells/mL were treated for several periods of time (as indicated) with jacalin (50 μg/mL) or PMA (60 ng/mL). Stimulation was stopped by a 13,000 × *g* centrifugation for 1 min at 4°. The cells were then lysed for 30 min on ice in lysis buffer (20 mM Tris pH 7,5; 140 mM NaCl; 2 mM EDTA; 50 mM NaF; 1 mM sodium orthovanadate; 1% Nonidet P-40 to which the protease inhibitors antipain, pepstatin, leupeptin (each at 2 μg/mL), aprotinin (10 μg/mL) and phenylmethysulfonyl fluoride (PMSF) (1 mM) were extemporaneously added. Lysates were clarified by centrifugation at 13000 × *g* for 20 min. 2-Mercaptoethanol-containing sample buffer (Laemmli) was then added and the samples were boiled and resolved in a 10% SDS-PAGE. After electrophoresis, proteins were transferred onto a PVDF membrane (polyscreen, NEN). Non-specific binding was blocked in PBS containing 5% BSA, 0.05% Tween 20; the membrane was then probed with the antiphosphotyrosine antibody 4G10 (1 μg/mL) and revealed with 1:10,000 solution of horseradish peroxidase-conjugated anti-mouse Ab followed by enhanced chemiluminescence detection system (NEN, Du Pont de Neumours) according to the manufacturer's instructions, on Reflection NEF films (NEN). Reprobing of the same blots with anti-ERK-2 Ab (0.2 μg/mL) was performed after stripping of bound Ab by incubating the membrane in a stripping buffer (6.25 mL of M Tris-HCl pH 6.8, 704 μL 2-ME, 10 mL 20% SDS, 83 mL H_2O) for 30 min at 50° followed by washing and resaturating in BSA containing solution. The membrane was revealed with horseradish-peroxidase anti-rabbit antibody (1:20,000) and the chemiluminescence detection system. Direct revelations (not stripped membranes) of ERK-2 and Raf-1 were realized on PVDF membranes electroblotted from 13% and 8% SDS-PAGE, respectively. The detection procedure was the same as that described for stripped membranes (anti-Raf-1 antibody was used as 0.5 μg/mL).

MAP Kinase Assays

GST-ELK-1 AS SUBSTRATE. 5.10^6 activated cells were lysed in 200 μL of cell extract buffer (25 mM Hepes pH 7.7, 0.3 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% Triton X-100, 20 mM β-glycerophosphate, 0.1 mM Na_3VO_4, 2 μg/mL leupeptin, 10 μg/mL benzamidin, 2 μg/mL aproti-

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nin, 1 μg/mL pepstatin, 100 μg/mL PMSF). After centrifugation, the supernatant was diluted with 600 μL dilution buffer (20 mM Hepes pH 7.7, 2.5 mM $MgCl_2$, 0.1 mM EDTA, 0.05% Triton X-100, 20 mM β-glycerophosphate, 0.1 mM Na_3VO_4, 2 μg/mL leupeptin, 10 μg/mL benzamidin, 2 μg/mL aprotinin, 1 μg/mL pepstatin, 100 μg/mL PMSF), incubated on ice for 10 min, and centrifuged again. Lysates were then mixed with 8 μg GST-fusion protein kinase substrate and glutathione-agarose (20 μL, Sigma) and incubated overnight at 4°. Experiments were performed as described [21] with GST-Elk-1 obtained from Dr. A. Nordheim. The substrate-agarose complexes were washed four times with binding buffer (20 mM Hepes pH 7.7, 50 mM NaCl, 25 mM $MgCl_2$, 0.1 mM EDTA, 0.05% Triton X-100), and *in vitro* phosphorylation was carried out for 20 min at 30° in the presence of 20 mM Hepes pH 7.6, 20 mM $MgCl_2$, 2 mM dithiothreitol, 20 mM β-glycerophosphate, 0.1 mM Na_3VO_4, 20 mM p-nitrophenyl phosphate, 20 μM ATP (4 μCi of [γ-^{32}P]ATP) [total volume: 30 μL]. The reaction was stopped by a single wash with binding buffer and by adding 30 μL of 2× Laemmli buffer. Proteins were fractionated by 10% SDS-PAGE, electrotransferred to PVDF membrane and subjected to autoradiography or quantitatively analysed with a PhosphorImager (Molecular Dynamics, Inc.).

MBP AS SUBSTRATE. 5.10^6 cells treated with jacalin (50 μg/mL) or PMA (60 ng/mL) were lysed in 1 mL lysing buffer. After centrifugation, lysates were clarified with protein A sepharose for 30 min and ERK-2 was immunoprecipitated. Briefly, clarified lysates were incubated overnight with 1 μg anti-ERK antibody at 4°. Protein A was added for 1 hr. The ERK-2/ anti-ERK-2 protein complexes were washed four times with lysis buffer and once with kinase buffer (10 mM HEPES, pH 7.4, 50 mM NaCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 nM Na_3VO_4). *In vitro* phosphorylation was carried out for 30 min at 30° in 50 μL kinase buffer with 10 μg MBP, 50 μM ATP, 1 μCi [γ-^{32}P] ATP. The reaction was stopped by adding 50 μL of 2× Laemmli buffer and boiling. Proteins were resolved in 10% SDS-PAGE, electroblotted to PVDF membrane and visualized by autoradiography and quantitatively analysed with a PhosphorImager.

RESULTS

Induction by Jacalin of Tyrosine Phosphorylations of Lymphocyte Intracellular Proteins Including the MAP Kinase ERK-2

$CD4^+$ purified human T lymphocytes were stimulated with PMA or with an optimal mitogenic concentration of jacalin for several periods of time (5, 15 and 20 min). Phosphorylation analysis from the cell lysates revealed (Fig. 1A) that several proteins were phosphorylated on tyrosine residues upon PMA and jacalin stimulation. The profile of the bands obtained from activation by the two mitogens was, however, quite different. It is noteworthy that the

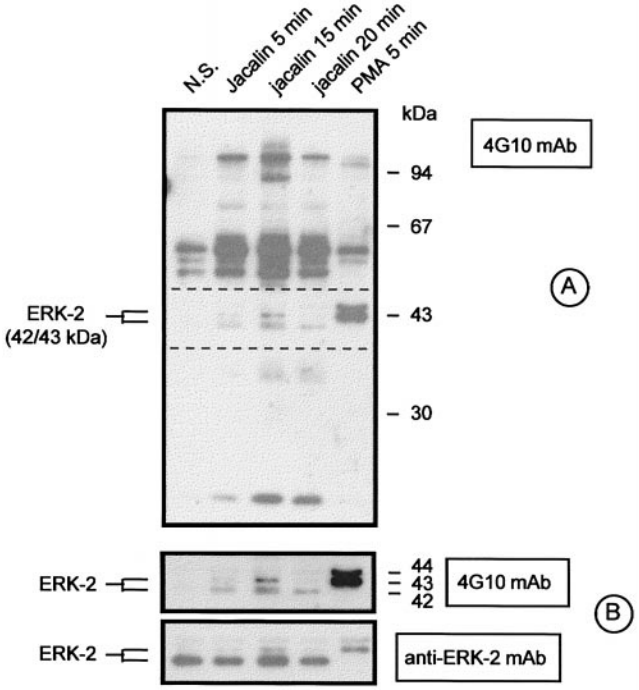

FIG. 1. Tyrosine phosphorylation of cellular proteins upon jacalin stimulation. (A) $CD4^+$-purified T cells were not stimulated (n.s.) or stimulated with jacalin for 5, 15 or 20 min or with PMA (5 min) used as a positive control. After cell lysis, the supernatants were run on SDS-PAGE and the gel electroblotted on nylon sheet. The membrane was then probed with mouse antiphosphotyrosine mAb (4G10) followed by horseradish peroxidase-conjugated anti-mouse antibody. Immunoreactive tyrosine phosphoproteins were visualized using chemiluminescence detection and film revelation. (B) The membrane stripped of bound antibody by incubation in a stripping buffer was reprobed with rabbit anti-ERK-2 antibody followed by horseradish peroxidase-conjugated anti-rabbit antibody. The ERK-2 corresponding bands were visualized as in (A) using the chemiluminescence detection system. The 4G10 revelation presented in Fig. 1B corresponds to the dashed squared marked out in Fig. 1A. This is a representative experiment out of four.

intensity of the jacalin-induced phosphorylated bands was maximum after 15 min stimulation. Stripping of the antiphosphotyrosine mAb from the electroblotted membrane and reprobing it with anti-ERK-2 mAb allows the identification of the tyrosine-phosphorylated 42 kDa bands (noted by a dashed square in Fig. 1A) as ERK-2 MAP kinase. For easier comparison of the bands, the dashed square is reproduced in Fig. 1B. In the anti-ERK-2 revealed membrane, a shifted band could be seen after 15 min stimulation. This shifted band was very weak after 5 min and almost disappeared after 20 min. As a positive control, PMA stimulation led to a complete shifted form of the band as previously described [22, 23]. 4G10 revealed an additional phophorylated band (44 kDa) in PMA stimulation which likely corresponds to ERK-1 and which was slightly revealed by cross-reaction with anti-ERK-2 antibody. Even though there was almost no visible shifted form of ERK-2 at 5 min, the kinase already appeared phosphorylated on tyrosine residues as revealed by 4G10 antiphosphotyrosine mAb.

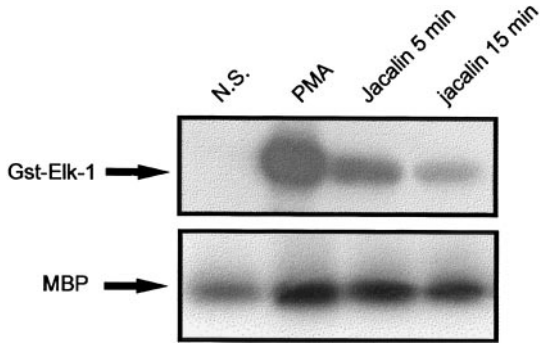

FIG. 2. Phosphorylation of GST-Elk-1 and MBP by activated ERK-2. Lysates from PMA or jacalin-activated or nonactivated (n.s.) cells were mixed with GST-Elk-1 and glutathione agarose overnight at 4°. *In vitro* phosphorylation was carried out in phosphorylation buffer in the presence of [γ^{32}P]-ATP. The proteins separated on 10% SDS-PAGE and electroblotted on nylon sheet were visualized by autoradiography. When MBP was used as substrate, ERK-2 was first immunoprecipitated from cell lysates. The immunoprecipitated ERK-2 was mixed in phosphorylation buffer with MBP and [γ^{32}P]-ATP. The proteins were separated on 10% SDS-PAGE, electroblotted on PVDF membrane and visualized by autoradiography. Quantitative analysis using a PhosphorImager gave the following values: n.s. 100%; PMA 167%; jacalin 5 min 160%; jacalin 15 min 137%. This experiment repeated twice led to the same result.

Time-Course Study of the ERK-2 Kinase Activity Induced by Jacalin

As shown in Fig. 1B, activation of ERK-2 was accompanied by its phosphorylation, which led to a shifted form of the kinase. However, if this does reflect an activated state of the kinase, it does not show actual enzyme activity. We therefore studied the phosphorylation (using ATPγ^{32}P) of myelin basic protein (MBP) as exogeneous substrate and of Elk-1 (the physiological substrate of ERK-2) (for easy recovering of the substrate we used the fusion protein Gst-Elk-1). ERK-2 activity through its ability to phosphorylate MBP or Gst-Elk-1 was studied. Estimation of the relative intensity of the phosphorylated MBP or Gst-Elk-1 bands (Fig. 2) shows that even though the degree of tyrosine phosphorylation of ERK-2 was more intense after 15 min stimulation, the enzyme activity appeared higher after 5 min than after 15 min.

Effect of Jacalin Stimulation of Raf-1 Phosphorylation

The preceding results showed that jacalin stimulation of purified CD4$^+$ lymphocytes led to activation of the MAP kinase ERK-2. ERK-2 is activated downstream p21Ras relayed through Raf-1 and MEK-1 activation [9, 10]. To confirm the involvement of this kinase cascade in jacalin stimulation, we studied the effect of the lectin on the phosphorylation of the MAPKKK Raf-1. As can be seen in Fig. 3, after 15 min stimulation a slow migrating band was revealed with an anti-Raf-1 antibody, indicating that Raf-1 was also phosphorylated upon jacalin stimulation.

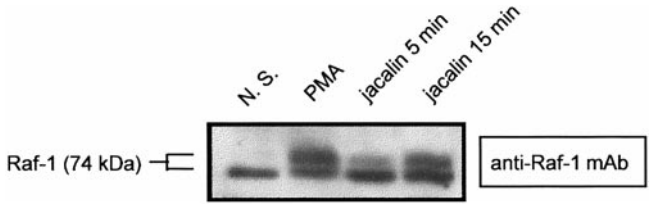

FIG. 3. Phosphorylation of Raf-1. A-CD4$^+$-purified T cells were not stimulated (n.s.) or stimulated with jacalin for 5 or 15 min or with PMA (5 min) used as a positive control. After cell lysis, the supernatants were run on SDS-PAGE and the gel electroblotted on nylon sheet. The membrane was then probed with rabbit anti-Raf-1 antibody followed by horseradish peroxidase-conjugated anti-rabbit antibody. Starting and shifted forms of Raf-1 were visualized using chemiluminescence detection and film revelation. This is a representative experiment out of four.

Evidence of the Dependence of IL2 mRNA Expression Induced upon Jacalin Stimulation in T Lymphocytes on the Activation of the Raf-1/MEK-1/ERK-2 Pathway

We studied in parallel the activation of ERK-2, evaluated through its phosphorylation state (shift of the ERK-2 electrophoretic band), and the expression of IL2 mRNA. Because complete jacalin-induced T cell stimulation leading to cell proliferation occurs only in the presence of autologous monocytes [14], the experiments were performed with CD4$^+$ cell-enriched lymphocytes supplemented with 20% monocytes; the cells were stimulated with jacalin or PHA used as positive control. Parallel experiments were performed with cells previously treated with PD098059, a specific inhibitor of the MAPKK MEK-1 which prevents the downstream phosphorylation and activation of ERK-2 [23, 24]. Figure 4 shows that upon treatment with PD098059 no shifted form of ERK-2 could be visualized from jacalin-stimulated CD4$^+$ cells. In parallel, no IL-2 mRNA could be detected in these cells pretreated with the MEK-1 inhibitor. This result demonstrates that jacalin-induced stimulation of CD4$^+$ lymphocyte involved activation of the MAP-kinase cascade and suggests that activation of this pathway was essential for the induction of proliferation. It must be pointed out that PD098059 did not completely inhibit stimulation of ERK-2 phosphorylation and IL2 messenger expression induced by PHA.

DISCUSSION

In the present paper, we show that stimulation of CD4-purified T cells from human peripheral blood by the lectin jacalin leads to phosphorylation of cellular proteins on tyrosine residues, including the ERK-2 MAP kinase. After 15 min stimulation with the lectin, a slow migrating form of ERK-2 corresponding to a hyperphosphorylated state of the kinase could be visualized. After 5 min, the slow migrating band was very faint; however, it corresponds to a higher activity of the enzyme as assessed by the degree of phosphorylation of Gst-Elk-1 or MBP used as exogeneous substrates. This could be explained by the fact that after 15

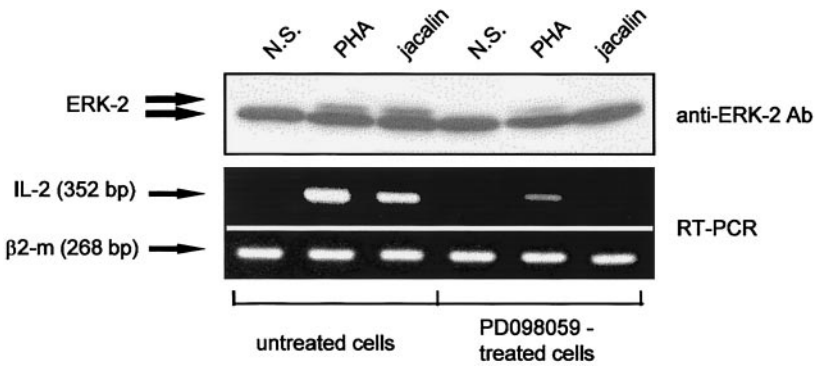

FIG. 4. Phosphorylation of ERK-2 and IL-2 messenger expression in cells stimulated with PHA or jacalin in the presence or absence of PD098059. $CD4^+$-purified T cells were not stimulated (n.s.) or stimulated with jacalin or with PHA in the presence or absence of the MEK-1 inhibitor PD098059. After cell lysis, the supernatants were run on SDS-PAGE and the gel electroblotted on nylon sheet. The membrane was then probed with rabbit anti-ERK-2 Ab followed by horseradish peroxidase-conjugated anti-rabbit antibody. The starting and shifted forms of ERK-2 were visualized using chemiluminescence detection and film revelation. In parallel, $CD4^+$ cells from the same preparation, supplemented with autologous monocytes, were stimulated for 6 hr with PHA or jacalin in the presence or absence of PD098059. Total RNA were submitted to RT-PCR analysis using IL-2 primers; mRNA β2 microglobulin was studied in parallel as standard control. This experiment was repeated twice.

min, the kinase can be optimally phosphorylated on other residues (Tyr and Ser/Thr) than those normally involved in enzymatic activity, leading to a higher proportion of the slow migrating form of the enzyme. This hypothesis is supported by the data of Krishnan *et al.* [25] who, using ERK-2 mutants, have shown that there is not an obligatory close correlation between the degree of phosphorylation of ERK-2 and the degree of its activity.

Activation of the MAP kinase pathway induced upon jacalin stimulation is confirmed by the phosphorylation of MAPKKK Raf-1 visualized by the appearance of a shifted electrophoretic band. Several studies have shown that activation of nuclear factors regulating IL2 gene transcription in T cells is dependent on the activation of the MAP kinase pathway [11, 12]. However, a recent study using primary T lymphocytes from transgenic mice expressing a mutated nonactive form of MAPKK MEK-1 has shown that these cells can still be stimulated, leading to a normal release of interleukin-2, providing evidence that the MAP kinase pathway could not be involved [13]. We have also shown (unpublished data) that stimulation of human primary T cells through the TcR/CD3 complex could occur independently of the activation of the Raf-1/MEK-1/ERK-2 pathway. The results presented here are lines of evidence that the lectin jacalin activates the MAP kinase pathway in primary T cells. Moreover, this activation appears to be essential for IL2 gene transcription, since the blockade of MAPKK MEK-1 by PD 098059, a specific inhibitor of this enzyme [23, 24], is accompanied by the total inhibition of IL2 mRNA expression. Because IL2 release from jacalin-stimulated lymphocytes requires the presence of autologous monocytes [15], it cannot be totally excluded that PD098059 may affect the accessory function of the monocytes in addition to inhibiting jacalin-triggered signaling T cells. However, this hypothesis appears unlikely, since it has been shown using separate chambers that monocytes play a role in the stimulation process through their physical interaction with lymphocytes rather than through synthesized and released molecules [15]. It must be noted that when the cells were stimulated with PHA, phosphorylation of the MAP kinase ERK-2 could not be totally inhibited by the blockade of MEK-1. Of course, this difference could be explained by the fact that PHA triggers a very intense activation as assessed by the high level of IL2 mRNA (Fig. 4) which cannot be blocked by the MEK inhibitor; this appears unlikely since the shift of ERK in PHA stimulation is very similar to that induced by jacalin. It is noteworthy that PHA stimulates the general T cell population, while jacalin has a narrow mitogenic specificity towards the CD4-expressing T lymphocytes [14, 15]. A possibility therefore exists that PHA activates another pathway in addition to the MAP kinase cascade, which could also lead to the phosphorylation of ERK-2. Such a Raf-1-independent phosphorylation pathway of ERK has already been suggested elsewhere [26]. This result could also be related to an earlier result showing that NF-AT activation is not totally abolished in Jurkat cells transfected with a plasmid encoding for a dominant negative MEK-1 mutant [27]; the same study demonstrates that the complete activation of the NF-AT transcription factor required the activation of a parallel pathway involving Rac-1.

Altogether, these results show that jacalin induces IL2 gene transcription in primary T lymphocytes through the activation of the Raf-1/MEK-1/ERK-2 pathway. Activation of this pathway is essential for IL2 gene transcription since its blockade abrogates IL2 mRNA expression. Jacalin stimulation thus appears as a good model for the study of the specific involvement of the MAP kinase pathway in $CD4^+$ T lymphocyte activation and for the screening of specific MAP kinase targeted drugs which could act on T cell proliferation.

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