We have demonstrated previously that microtubule depolymerization by colchicine in human monocytes induces selective production of interleukin-1 (IL-1) (Manié, S., Schmid-Alliana, A., Kubar, J., Ferrua, B., and Rossi, B. (1993) J. Biol. Chem. 268, 13675–13681). Here, we provide evidence that disruption of the microtubule structure rapidly triggers extracellular signal-regulated kinase (ERK) activation, whereas it was without effect on SAPK2 activity, which is commonly acknowledged to control pro-inflammatory cytokine production. This process involves the activation of the entire cascade including Ras, Raf-1, MEK1/2, ERK1, and ERK2. Activation of ERKs is followed by their nuclear translocation. Although other SAPK congener might be activated upon microtubule depolymerization, the activation of ERK1 and ERK2 is mandatory for IL-1 production as shown by the blocking effect of PD 98059, a specific MEK1/2 inhibitor. Additionally, we provide evidence that microtubule disruption also induces the activation of c-Src and Hck activities. The importance of Src kinases in the mediation of the colchicine effect is underscored by the fact that CP 118556, a specific inhibitor of Src-like kinase, abrogates both the colchicine-induced ERK activation and IL-1 production. This is the first evidence that ERK activation is an absolute prerequisite for induction of this cytokine. Altogether, our data lend support to a model where the status of microtubule integrity controls the level of Src activities that subsequently activate the ERK kinase cascade, thus leading to IL-1 production.

Monocytes play an essential role in the inflammation as accessory cells for the processing and presentation of antigen to lymphocytes, but also in the inflammatory process by releasing oxygen metabolites, lysosomal enzymes, and cytokines such as tumor necrosis factor-α and interleukins (ILs)1 1 and 6. The regulation of the synthesis of these cytokines is complex, and still poorly understood. Bacterial endotoxin (lipopolysaccharides), antigen-antibody complexes, phorbol esters (phorbol 12-myristate 13-acetate), and cytokines are classical monocyte-activating agents which induce the concerted production of these pro-inflammatory cytokines. Recent studies have shown that alteration of the cytoskeletal network by chemical agents also induces a dramatic and specific increase of IL-1 (1, 2) and tumor necrosis factor-α (3, 4) by monocytes.

During recent years, growing evidence has accumulated showing that the cytoskeleton could intervene in the propagation of the mitogenic and activation signal (5). In this respect, many studies have focused on the actin network that acts as a dynamic structure involved in the integrin-mediated signaling cascade (6). Cytoplasmic microtubules represent another major element of the cytoskeleton that have been implicated in diverse processes such as cellular motility, intracellular transport, and secretion. The fact that microtubules are subject to constant remodeling, because of the dynamic instability of tubulin dimers, prompted us to consider that the microtubule network may be an important actor in the transmission of activation signals inside the cell. This idea is supported by reports showing that: (i) microtubule reorganization, occurring during differentiation of HL 60 cells, is associated to tubulin phosphorylation on tyrosine residues (7); (ii) microtubule reorganization were also observed after cytokines and phorbol ester treatment of human umbilical vein endothelial cells (8); and (iii) microtubule disruption generates a signal that leads to NFκB activation (9).

We and others have shown that microtubule-disrupting drugs are capable of generating a signal that leads to the selective induction of IL-1 synthesis in human monocytes (1, 2). We demonstrate that microtubule depolymerization was without effect on SAPK2 activity, which is generally considered as a key regulating enzyme in the production of cytokines (10, 11). In contrast, we provide the first evidence that the colchicine-mediated microtubule disassembly can induce, by itself, the activation of the entire Ras-dependent cascade leading to ERK activation and their translocation to the nucleus. This cascade is in fact triggered by the upstream transient activation of c-Src and Hck. Owing to the selective effect of colchicine on IL-1 production, this study emphasizes the importance of the microtubule polymerization state in the control of the regulation it exerts on some Src-like kinases.

EXPERIMENTAL PROCEDURES

Cell Culture and Human Monocyte Preparation

Human myelomonocytic THP1 cells (ATCC) were grown in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal calf serum (Biooptica), 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (referred to as the complete medium). Fetal calf serum was tested for the absence of endotoxin (<0.1 IU/ml, Institut
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J. Boy, Reims, France. Cells were maintained at 37 °C in a humidified 5% CO2 atmosphere.

Human peripheral blood mononuclear cells were isolated under sterile conditions from leukopheresis samples obtained from the Center de Transfusion Sanguine A. Tzanev (St. Laurent du Var, France) and treated with the anti-SRC monoclonal antibody Tyr-527 (370 kDa) (1). Mononuclear cells were cultured for 8 h to allow adherence-induced transcription of IL-1 mRNA to subside.

Measurement of IL-1β Production

Human monocytes (5 × 10^6 cells/ml) were stimulated for 18 h in RPMI 1640 medium and then harvested by a 5-min centrifugation at 1000 × g before being resuspended in RPMI-Hepes (Life Technologies, Inc.) at a concentration of 2 × 10^6 cells/ml. Cells (5 × 10^5) were treated with or without the effectors for 3 h at 37 °C. Nuclei were isolated from these cells, and the run-on transcription assay was performed as described previously (12).

Nuclei Isolation and Run-on Transcription Assay

Human monocytes or THP1 cells (7 × 10^5 cells/ml) were starved 16 h in RPMI 1640 medium and then harvested by centrifugation for 5 min at 1000 × g before being resuspended in RPMI-Hepes (Life Technologies, Inc.) at a concentration of 2 × 10^6 cells/ml. Cells (10 × 10^5) were treated with or without the effectors for the indicated times at 37 °C and lysed in buffer (150 mM NaCl, 0.8 mM MgCl2, 5 mM EGTA, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 15 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM Na3VO4, and 50 mM Hepes, pH 7.5). The crude lysates were centrifuged at 18,000 × g for 20 min at 4 °C, and the supernatants were precleared with nonimmune serum prebound to protein A-Sepharose (Pharmacia Biotech Inc.) for rabbit serum or protein G-Sepharose (Santa Cruz Biotechnology). The immunopellets were washed twice with lysis buffer and twice with MAP kinase buffer (30 mM NaCl, 10 mM MgCl2, 1 mM dithiothreitol, 10 mM β-nitrophenylphosphate, 30 mM Hepes, pH 7.5). In parallel, using specific antibodies, we immunoprecipitated ERK1, from unstimulated THP1 lysates, to be used as a substrate. The pellets containing MEK and ERK1, respectively, were then mixed in a final volume of 50 μl of MEK kinase buffer in the presence of 15 μM ATP. The reaction was started by addition of 100 μCi/ml [γ-32P]ATP. After addition of 25 μl of 9 × Laemmli sample buffer to stop the reaction, the samples were heated to 95 °C for 3 min. Samples were split to be analyzed by SDS-PAGE. Gels were either exposed for autoradiography using hyperfilms (Amersham) or probed with antibodies for immunoblotting experiments. Autoradiographies were scanned using a Ultrorad screen (Amersham). Anti-c-Src and anti-Hck antibodies were from Santa Cruz Biotechnology. Pervanadate was prepared as described previously (14).

Western Blotting Analysis

Total cell lysates (100 μg), immunoprecipitated substrates, or nuclear proteins (100 μg) were separated by SDS-PAGE and transferred to Immobilon membrane as detailed previously (15). The blots were probed with 4G10 anti-phosphotyrosine (Upstate Biotechnology, Inc., Lake Placid, NY) or anti-c-Src at 1 μg/ml, or with anti-ERK1, anti-ERK2, anti-SAPK2/P38, anti-Raf-1, anti-MEK-1, or anti-Hck (Santa Cruz Biotechnology) at 0.1 μg/ml, the proteins were visualized by the Amersham ECL system and quantified by densitometric scanning.

Immune Complex Kinase Assay

Src Kinase Activity—The Src-related kinases were immunoprecipitated with anti-c-Src antibodies (Santa Cruz Biotechnology) bound to protein G-Sepharose or with anti-Hck antibodies (gift from I. Maridon-neau-Parini) bound to protein A-Sepharose. The immunoprecipitates were washed twice with lysis buffer, followed by one wash with lysis buffer supplemented with 0.25%/deoxycholate, and ultimately washed twice in Src kinase buffer (5 mM MgCl2, 5 mM MnCl2, 30 mM Hepes, pH 7.5). Samples were then resuspended in 50 μl of Src kinase buffer supplemented with 0.1 mg/ml acid-denatured enolase, which was used as an exogenous substrate. The kinase assay was started by addition of 40 μCi/ml [γ-32P]ATP (370 MBq/ml, Amersham Life Science). After addition of 25 μl of 9 × Laemmli sample buffer to stop the reaction, samples were heated to 95 °C for 3 min. Samples were split to be analyzed by SDS-PAGE. Gels were either exposed for autoradiography using hyperfilms (Amersham) or probed with antibodies for immunoblotting experiments. Autoradiographies were scanned using a Ultrorad screen (Amersham). Anti-c-Src and anti-Hck antibodies were from Santa Cruz Biotechnology. Pervanadate was prepared as described previously (14).

MAP Kinase Activity—MAP-related kinases were immunoprecipitated with anti-SAPK2/P38, anti-ERK1, or anti-ERK2 antisera (Santa Cruz Biotechnology) bound to protein A-Sepharose. The immunoprecipitates were washed twice with lysis buffer, twice with MAP kinase buffer (30 mM NaCl, 0.1% Nonidet P-40, 10% glycerol, 200 μg/ml Na3VO4, 30 mM Hepes, pH 7.5), and resuspended in 50 μl of MAP kinase buffer in the presence of 75 μM ATP, 30 mM Mg acetate, and 0.2 mM/ml myelin basic protein (MBP, Sigma), which was used as an exogenous substrate. The kinase assay was initiated by addition of 100 μCi/ml [γ-32P]ATP. After addition of 25 μl of 9 × Laemmli sample buffer to stop the reaction, the samples were heated to 95 °C for 3 min. Samples were split to be analyzed by SDS-PAGE. Gels were either exposed for autoradiography using hyperfilms (Amersham) or probed with antibodies for immunoblotting experiments. Autoradiographies were scanned using a Ultrorad screen (Amersham). Anti-c-Src and anti-Hck antibodies were from Santa Cruz Biotechnology. Pervanadate was prepared as described previously (14).
Microtubule Disruption Activates Src and Hck Activities

FIG. 1. Measurement of the transcriptional activity of IL-1β gene in isolated nuclei from human monocytes exposed to colchicine. Human monocytes (3 × 10^6 cells) were exposed for 3 h in the absence of effector (lane 1), or with 1 μM lumicolchicine (lane 2) or 1 μM colchicine (lane 3). Nuclei were isolated and [α-32P]UTP was incorporated into nascent RNA chains as described under “Experimental Procedures.” Labeled RNAs (10^5 cpm) were then hybridized to IL-1β or GAPDH cDNA previously immobilized onto nitrocellulose filters and exposed to X-ray film for autoradiography.

human monocytes previously stimulated for 3 h with colchicine or lumicolchicine. Labeled RNAs were then hybridized to nitrocellulose filters previously spotted with plasmids harboring either the IL-1β or the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) coding sequence. A detectable constitutive level of IL-1β transcription was observed (Fig. 1, lane 1), but the treatment with colchicine dramatically increased the IL-1β transcription rate (Fig. 1, lane 3). Under the same conditions, lumicolchicine, the inactive analog of colchicine, was without any effect (lane 2). The slight inhibition that lumicolchicine induced on IL-1β transcription was not significant when normalized against the corresponding GAPDH spot. These results suggest that disruption of the microtubule network increased pro-IL-1β mRNAs at the level of transcription.

Microtubule Depolymerization Does Not Increase SAPK2/P38 Activity but Does Stimulates the Kinase Activity of ERK1 and ERK2 and Causes Their Nuclear Translocation—It has been reported that microtubule-disrupting agents, such as colchicine or microtubule-stabilizing agents, e.g. taxol, could modulate tyrosine kinase activities in intact cells (4, 16, 17). On the basis of these observations, we hypothesized that tyrosine kinase activities might participate in colchicine-induced IL-1β production. We first tested the effects of herbimycin A and genistein, two potent tyrosine kinase inhibitors, on the colchicine-induced IL-1β production in human monocytes. As shown in Fig. 2A (upper panel), herbimycin A and genistein both inhibited colchicine-induced IL-1β production, with ID₅₀ values of 10 nM and 5 μM, respectively. Conversely, as shown in Fig. 2A (lower panel), the colchicine-induced IL-1β production was dramatically increased in the presence of pervanadate, a potent inhibitor of tyrosine phosphatases (14) with a maximal synergizing effect at 30 μM. Similar effects of herbimycin A, genistein, and pervanadate were obtained when the production of the IL-1α protein was assayed instead of IL-1β (data not shown). It is noteworthy that herbimycin A does not prevent colchicine-induced cAMP accumulation in human monocytes (data not shown), suggesting that the colchicine-induced CAMP response, as reported previously (18, 19), is regulated by a pathway distinct from that controlled by the herbimycin A-sensitive tyrosine kinase activity.

In response to microtubule network disruption, the activation of a tyrosine kinase activity was confirmed by the detection of phosphorytrosine proteins following colchicine treatment. As shown in Fig. 2B, exposure of THP1 cells to colchicine resulted in an increased tyrosine phosphorylation of five proteins with apparent molecular masses of 27, 28, 42, 55, and 60 kDa, respectively. The presence of tyrosine-phosphorylated protein(s) migrating in the 42–44-kDa range prompted us to investigate the possibility that MAPK congener(s) were activated upon microtubule disruption.

We focused first our attention on SAPK2/p38 activity, which has been demonstrated to play a key role on the IL-1 production induced by LPS stimulation (10, 11). Anti-SAPK2/p38 antibodies were used to isolate this type of MAPK from lysates of cells exposed or not to colchicine. The immunoprecipitates were then incubated with [γ-32P]ATP and MBP, a standard substrate for MAPKs, to assess their phosphotransferase activity. Under these conditions, we failed to detect any increase of SAPK2 activity in the presence or in the absence of colchicine.
Microtubule Disruption Activates Src and Hck Activities

Microtubule disruption stimulates ERK activities and causes their nuclear translocation in THP1 cells. A, time course of colchicine-induced stimulation of ERK activities. Cells were untreated (lane 1) or stimulated for 1 min (lane 2), 5 min (lane 3), 20 min (lane 4), 30 min (lane 5), 45 min (lane 6), or 60 min (lane 7) with 1 μM colchicine. ERK1 or ERK2 were immunoprecipitated using specific antibodies, and the kinase activities of immunoprecipitated ERK1 (upper box) or ERK2 (lower box) were assessed using exogenous MBP as substrate. B, densitometric scanning of ERK1 (A) and ERK2 (B) activities toward MBP. The bands corresponding to MBP phosphorylation were subjected to densitometric scanning and expressed as -fold stimulation of the basal level determined in unstimulated THP1 cells. Each value was corrected for small variations in the amounts of ERK1 or ERK2 present in each immunopellet, as assessed by Western blotting analysis. C, kinetics of ERK translocation to the nucleus. Western blot analysis of the nuclear content of phospho-ERK1 and phospho-ERK2 was estimated after exposure of THP1 cells to 1 μM colchicine for 0 min (lane 1), 5 min (lane 2), 10 min (lane 3), 20 min (lane 4), 40 min (lane 5), 60 min (lane 6), 90 min (lane 7), and 120 min (lane 8). Nuclear proteins (100 μg) were separated by SDS-PAGE, and transferred onto nitrocellulose membrane as described under “Experimental Procedures.” Immunoblots were then either probed with a specific anti-phospho-ERK antibody (upper box) or a specific anti-α-tubulin antibody (lower box), and specific bands were revealed by enhanced chemiluminescence.

(Fig. 3A). It is noticeable that the high basal activity was not inhibited by the addition of SB 203580, a specific inhibitor of SAPK2a and SAPK2b activities (11), highly suggesting that these two activities were not activated upon microtubule disruption. This was confirmed by the lack of effect of SB 203580 on colchicine-induced IL-1β production, as evidenced in Fig. 3B.

We then envisaged that other congeners of the MAPK family could participate to the colchicine induction of IL-1 synthesis. To this end, anti-ERK1 and anti-ERK2 antibodies were used to immunoprecipitate the serine/threonine kinases from untreated or colchicine-treated cell lysates. The immunopellets were then incubated with [γ-32P]ATP and MBP. The data presented in Fig. 4 demonstrate that the treatment of THP1 cells with colchicine increases ERK1 (Fig. 4, A (upper) and B) and ERK2 (Fig. 4, A (lower) and B) kinase activities. Indeed, microtubule depolymerization promoted a swift activation of ERK2, which culminated at 2 min and then declined to the basal level by 60 min. In the case of ERK1, kinase activity was maximal by 5 min after colchicine treatment and persisted for 60 min, at variance with ERK2. We verified that lumicolchicine failed to activate any ERK activities (data not shown).

MAPKs have been shown to phosphorylate and thereby to activate many regulatory proteins located in diverse cellular compartments, including nuclear transcriptional factors (20, 21). The nuclear content of phosphorylated MAPKs from cells treated or not with colchicine were assessed by Western blot analysis (Fig. 4C, upper box), using antibodies recognizing the phosphorylated forms of ERK1 or ERK2. Following microtubule disruption, ERK1 and ERK2 translocated to the nucleus 10 min (lane 2) after colchicine treatment, according to their activation profile (Fig. 4, A and B). Augmentation of activated ERK in the nuclear compartment did not result from contaminating cytosolic proteins since the tubulin level, or Sos (data not shown) used as reporter proteins, remained constant in all conditions (Fig. 4C, lower box).

Activation of ERK1 and ERK2 seems to be mandatory for mediating the colchicine effect since PD 98059, a blocker of MEK-1 and MEK-2 (22), abolished the stimulating effect that colchicine elicits on IL-1β production (Fig. 5, upper) and on IL-1β mRNA transcription (Fig. 5, lower) with an IC50 of 1 μM.

Colchicine Activates MEK and Raf-1 Kinase and Stimulates Nucleotide Exchange on p21ras—To understand the mechanisms by which microtubule depolymerization stimulated ERKs, we studied the colchicine effect on the upstream acting MEK and Raf-1 kinase activities.

It is well established that ERK1 and ERK2 are activated by the upstream MEK-1 and MEK-2 kinases (23, 24). The MEK-1 activity was measured in our system by mixing an anti-MEK-1 immunoprecipitate from colchicine-treated THP1 cell lysates with an immunopurified ERK1 inactive fraction obtained from unstimulated cell lysates. The data presented in Fig. 6A (upper panel) provide evidence that microtubule depolymerization (lanes 2–6) stimulates MEK-1 activity. Phosphorylation of ERK1 used as substrate rose steeply after 5 min of treatment, to culminate at 30 min, and then slowly declined by 60 min. It is noteworthy that the time course of ERK1 phosphorylation in response to colchicine treatment paralleled that of MEK-1 autophosphorylation (Fig. 6, lower panel).
Since Raf-1 activation sits upstream of the MEK activities, we sought to determine the colchicine effect on this activity. Therefore, Raf-1 was isolated from colchicine-treated THP1 cell lysates by using specific antibodies. Raf-1 kinase activity was then assessed by following phosphorylation of the unphosphorylated substrate MEK-1, obtained by previous immunoprecipitation from unstimulated THP1 cell lysates. A 6-fold increase in the extent of MEK phosphorylation was observed within 2 min following addition of Raf-1 immunoprecipitated from colchicine-treated cell lysates to prephosphorylated and unstimulated MEK. This activation lasted for at least 30 min (Fig. 6B).

The mechanism of Raf-1 activation has been studied extensively over the past few years, and it is now clear that p21ras plays a key role in Raf-1 activation (25). Raf-1 has been shown to be recruited to the plasma membrane by p21ras when the latter binds to GTP (26, 27). Thus, we determined whether colchicine was capable of modifying the relative level of p21ras bound to GTP. To this end, THP1 cells were labeled in vitro with [32P]orthophosphate, then subjected to colchicine treatment and lysed. Thereafter, p21ras was immunoprecipitated and the bound nucleotides were eluted and separated by thin layer chromatography. Under these conditions, we observed (Fig. 6C) in unstimulated cells (lane 1) that p21ras was mainly associated to GDP (97%) and under microtubule depolymerization the proportion of p21ras-GTP complex increased in a discrete but reproducible (n = 4) manner from 3% to 5%. This extent of stimulation was similar to that observed after activation of insulin (28), platelet-derived growth factor (29), or epidermal growth factor (30) receptors. The accumulation of p21ras associated to GTP was maximal after 2 min of colchicine treatment and then declined to the basal level by 5 min.

**Microtubule Depolymerization Stimulates the Kinase Activity of c-Src and Hck in Human Monocytic Cells**—As noted above (Fig. 2), the stimulation of human monocytic cells by colchicine resulted in an increased tyrosine phosphorylation of a protein set, including proteins in the 55–60-kDa range, which are reminiscent of Src kinase congeners (31–33). We thus examined the ability of colchicine to stimulate the kinase activity of four members of the Src family that are expressed in THP1 cells, namely pp60csrc, pp59hck, pp53/56lyn, and pp59lyn. The four kinases were immunoprecipitated with appropriate antibodies from unstimulated or colchicine-treated THP1 cell lysates and then tested for their ability to phosphorylate the exogenous substrate enolase in vitro and to undergo autophosphorylation. As shown in Fig. 7 (A and B), c-Src as well as Hck activities peaked at 5–10 min and subsided within 30 min following colchicine treatment. The extent of autophosphorylation of the two kinases in response to microtubule depolymerization paralleled their respective kinase activity toward enolase. Interestingly, activation of c-Src and Hck did not reflect a general increase in Src kinase activity, since Lyn activity remained unaffected, while Fyn activity was slightly diminished by the colchicine treatment (data not shown).

Involvement of Src kinases in mediating the colchicine effect is further supported by the finding that CP 118556, a potent inhibitor of the Src kinase family (34), inhibits the alkalioid-induced IL-1 stimulation, as depicted in Fig. 7C. Diminution of the IL-1β transcript level by CP 118556 was dose-dependent with an IC50 of 3 μM, in accord with the report of the effect of this compound on Src activities measured in intact cells (34). We verified that, under CP 118556 treatment, inhibition of c-Src activity (Fig. 8A) was correlated to a concomitant inhibition of the stimulation of ERK1 and ERK2 (Fig. 8B), indicating that Src kinases were involved in the up-regulation of ERK activities. Cell viability was not affected under these conditions, as assessed by trypan blue staining (data not shown).
Increased transcription rate of pro-IL-1β mRNA. These data suggest that microtubule depolymerization not only controlled events occurring in the cytoplasmic compartment, but also conveyed a signal to the nucleus resulting in the activation of transcription factors necessary for the expression of the IL-1β gene. Along this line, the recent report showing that microtubule depolymerization could activate NFκB in HeLa cells (9) is of particular interest, owing to the importance of this factor in the control of the IL-1β gene (36).

To gain information on the signal propagation from the microtubules to the nucleus, we focused our attention on the MAPK congeners that are known to play a key role in the activation of several transcription factors, including NFκB and NF-IL-6 (37, 38). Several features of MAPKs substantiate an inter-relationship between the activation of this kinase family and microtubule remodeling: (i) about 40% of ERK1 and ERK2 are associated to the microtubules (39); (ii) the best-characterized cellular factors that regulate microtubule dynamics are MAPs, phosphorylation of which by MAPKs has been proposed to play a major role in the microtubule growing/shrinking balance (40).

Owing to the important role that was ascribed to SAPK2/P38 activity in the control of the LPS-induced IL-1 production (11), we first investigated the possibility that this subfamily of MAPKs was also involved in the colchicine-induced IL-1 up-regulation. However, we found that no SB 203580-sensitive activity was recovered in anti-SAPK2/P38 immunopellets, ruling out a possible involvement of this type of activity in the mediation of the colchicine effect. In contrast, ERK1 and ERK2 were activated upon microtubule disruption following two distinct kinetic profiles. ERK2 appears to be activated transiently, while ERK1 was activated in a more sustained fashion. These kinetic profiles perfectly matched the respective translocation pattern of the two MAPK species to the nucleus. We demonstrated that not only ERK1 and ERK2 were activated, but activators situated upstream, such as MEK-1, Raf-1, and Ras, were also stimulated upon microtubule depolymerization with a kinetic profile compatible with that observed for ERK activation. cAMP-dependent protein kinase has been found to be activated upon microtubule disruption (1, 2), and this could lead to directly activate ERK activity as reported in some systems (41, 42). Our data suggest instead that the activating effect of colchicine on ERKs does stem from the activation of the entire MAPK cascade, rather than the mere activation of ERK via cAMP-dependent protein kinase. We cannot exclude the possibility that other SAPK congeners such as SAPK1/JNK1, SAPK3, and SAPK4 (43) are also activated in response to colchicine treatment. However, when THP1 cells were exposed to PD 98059, a specific inhibitor of MEK-1 (22), and by way of consequence a blocker of the ERK1/ERK2 pathway, we totally blocked the stimulatory effect of the vinca-alkaloid on the expression of IL-1β RNA. These data provide the first evidence that activation of ERK activities is a mandatory condition for the up-regulation of IL-1β transcription. This agrees well with the notions that (i) MAPK dramatically up-regulates NF-IL-6 transactivating activity through phosphorylation of its Thr-235 residue (37) and (ii) NF-IL-6 plays an essential role in the up-regulation of IL-1β gene by interacting with motifs situated on upstream and proximal regions of the promoter (44).

In an attempt to understand the mechanism by which microtubule disruption could activate Ras or acts on a step situated upstream, we investigated a possible involvement of tyrosine kinase activity(ies), which have been shown to act as potent activators of Ras through the Grb-2/Sos pathway (45, 46). This hypothesis was supported by (i) the observation that tyrosine kinase inhibitors such as herbimycin or genistein abol-
ished the colchicine-induced IL-1β transcription; and (ii) the fact that pervanadate, a potent inhibitor of tyrosine phosphatases, acts with the activating effect induced by suboptimal concentration of colchicine. When THP1 cells were exposed to colchicine, they exhibited a complex tyrosine phosphorylation pattern including a set of phosphoproteins migrating in the range of 55–60 kDa that was reminiscent of tyrosine kinases of the Src type. We could indeed demonstrate that both c-Src and Hck tyrosine kinase activities were transiently stimulated with a time course compatible to that observed for the activation of the MAPK cascade. Activation of c-Src and Hck was found to be of crucial importance for the mediation of the colchicine effect since addition of CP 118556, which inhibits Src-like kinase activities (34), also abrogated the increase in IL-1β transcription induced by microtubule disruption. When we looked to other members of the Src kinase family expressed in THP1 cells, we found that exposure to colchicine had no effect on the level of Fyn activity while that of Lyn was slightly decreased (data not shown), thus suggesting that, depending on their degree of assembly, tubulin dimers exert a fine regulation on the range of 55–60 kDa that was reminiscent of tyrosine kinase activities (34), also abrogated the increase in Fyn activity while that of Lyn was slightly decreased (data not shown), thus suggesting that, depending on their degree of assembly, tubulin dimers exert a fine regulation among the Src tyrosine kinase congeners. Although colchicine treatment does not correspond to a physiological condition, modulation of microtubule stability does occur under physiological or pathological conditions. For instance, adherence of avian macrophages to fibronectin or vitronectin-coated surfaces that is known to reorganize the microtubule network has been reported to induce the association of c-Src to polymerized tubulin in a tyrosine kinase independent manner (47). Additionally, we could demonstrate that pretreatment of THP1 cells with CP 118556 abrogated at the same time c-Src activity and ERK1 and ERK2 activities, strongly suggesting that, consecutively to microtubule disruption, c-Src acts as a positive regulator situated upstream of the ERK activities.

In conclusion, microtubule disruption by vinca-alkaloids appears as a valuable tool to decipher the pathways specifically involved in IL-1 gene regulation in human monocytes, which points to a major role played by the Src-triggered ERK cascade under colchicine treatment. Given that SAPK2 was shown to be essential for the production of the IL-1 under LPS stimulation, our data suggest that, depending on the site inflammatory stimulus, IL-1 production might be controlled by distinct MAPK congeners.

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