Role of Interleukin (IL)-2 Receptor β-Chain Subdomains and Shc in p38 Mitogen-activated Protein (MAP) Kinase and p54 MAP Kinase (Stress-activated Protein Kinase/c-Jun N-terminal Kinase) Activation

IL-2-DRIVEN PROLIFERATION IS INDEPENDENT OF p38 AND p54 MAP KINASE ACTIVATION*

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We have shown recently that interleukin (IL)-2 activates the mitogen-activated protein (MAP) kinase family members p38 (HOG1/stress-activated protein kinase) II and p54 (c-Jun N-terminal kinase/stress-activated protein kinase I). Furthermore, the p38 MAP kinase inhibitor SB203580 inhibited IL-2-driven T cell proliferation, suggesting that p38 MAP kinase might be involved in mediating proliferative signals. In this study, using transfected BA/F3 cell lines, it is shown that both the acidic domain and the membrane-proximal serine-rich region of the IL-2Rβ chain are required for p38 and p54 MAP kinase activation and that, as for p42/44 MAP kinase, this activation requires the Tyr392 residue of the acidic domain, the binding site for Shc. It is well established that the acidic domain of the IL-2Rβ chain is dispensable for IL-2-driven proliferation, and thus our observations suggest that neither p38 nor p54 MAP kinase activation is required for IL-2-driven proliferation of BA/F3 cells. In addition, the tetravalent guanylylhydrazone inhibitor of proinflammatory cytokine production, CNI-1493, can block the activation of p38 and p54 MAP kinases by IL-2 but has no effect on IL-2-driven proliferation of BA/F3 cells, activated primary T cells, or a cytotoxic T cell line. Furthermore, our observations provide evidence for the existence of an additional, unknown target of the p38 MAP kinase inhibitor SB203580, the activation of which is essential for mitogenic signaling by IL-2.

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†† The abbreviations used are: IL, interleukin; IL-2R, interleukin-2 receptor; MAP, mitogen-activated protein; MAPK, MAP kinase; GST-

in driving this process (1). The intracellular signal transduction pathways activated by IL-2 and the relative roles of these pathways in mediating the mitogenic signal have been extensively studied but have yet to be fully elucidated.

IL-2 exerts its cellular effects through binding to specific cell surface receptors. The high affinity IL-2 receptor (IL-2R) is a heterotrimeric complex consisting of α-, β-, and γ-, subunits, the γ- subunit being shared with the receptors for the other T cell mitogens, IL-4, IL-7, IL-9, and IL-15 (2). The α-subunit is responsible for conferring high affinity cytokine binding, while the β- and γ- subunits recruit cytoplasmic molecules, thereby transducing the proliferative signal. The β-subunit has the larger cytoplasmic tail, consisting of subdomains previously identified as the membrane-proximal serine-rich, acidic, and distal proline-rich regions (3, 4). The IL-2Rβ chain contains six cytoplasmic tyrosine residues: Tyr338, Tyr355, Tyr358, and Tyr361, which lie in the acidic region, and Tyr392 and Tyr510, which lie in the proline-rich region. The presence of at least one of the tyrosines Tyr338, Tyr355, and Tyr358 and Tyr392 appears to be sufficient to allow IL-2-driven proliferation (5, 6). Studies in transfected BA/F3 cells have shown that loss of the IL-2Rβ acidic region has no effect on proliferation (4) or the expression of Myc and Bcl-2, factors essential for proliferation and cell survival (7). The tyrosine kinases p56Lck, p72Syk, Jak1, and Jak3 are recruited to the IL-2R and activated upon IL-2 binding (8–11). However, of these only the activation of Jak3, which associates with the IL-2Rγ chain, appears to be an absolute requirement for IL-2-driven proliferation (8, 10, 12–16). It has been suggested that Jak3 mediates IL-2 proliferative signaling through activation of another tyrosine kinase, Pyk2 (17). Signal transducer and activator of transcription (STAT) 5 is also activated as a result of Jak activation, but its role in proliferation is unclear (6, 18, 19).

IL-2 activates two other major signaling pathways: the phosphatidylinositol 3-kinase pathway and the p42/44 MAP kinase pathway. The activation of phosphatidylinositol 3-kinase (20, 21) and the subsequent activation of protein kinase B/Akt (22) and p70S6 kinase (23, 24), has been associated with the serine-rich region of the IL-2Rβ chain (25), and the activation of these factors results in the phosphorylation of Rb, suggesting a key role for these kinases in proliferation (26). IL-2-induced activation of the p42/44 MAP kinase (extracellular signal-regu-
lated kinase) pathway proceeds through the activation of p21^{ras}, Raf, and MAP kinase kinase 1/2 (27–29) and requires both the acidic and the serine-rich regions of the IL-2R{\beta} chain (30). Tyr^{355}, within the acidic region of the IL-2R{\beta} chain, is responsible for the recruitment of Shc (31) and the subsequent assembly of the p21^{ras}-activating complex along with Grb2 and SOS (31–33). However, loss of p42/44 MAP kinase signaling does not appear to prevent IL-2-driven cell cycle progression (30, 31, 34). Two further MAP kinase family members, p54 (stress-activated protein kinase Ic-Jun N-terminal kinase) and p38 (stress-activated protein kinase II/HOG1), were typically thought to be activated by cellular stress and proinflammatory stimuli (35–39). However, we have recently demonstrated that p38 MAP kinase and p54 MAP kinase are activated by IL-2 in T cells (40). We observed that an inhibitor of p38 MAP kinase function, SB203580 (39), was able to inhibit T cell proliferation induced by IL-2, suggesting that p38 MAP kinase activation may be required for cell cycle progression.

We have now examined further the role of these kinases in IL-2 proliferative signaling by mapping the regions of the IL-2R{\beta} chain required for p38 and p54 MAP kinase activation. We observe that both the serine-rich and the acidic regions are required for the activation of p54 and p38 MAP kinases by IL-2 in BA/F3 cells and that the activation of these kinases, like that of p42/44 MAP kinase, is dependent on the presence of Tyr^{355} in the IL-2R{\beta} chain and proceeds through the recruitment of Shc. Since the acidic region of the IL-2R{\beta} chain is dispensable for BA/F3 cell proliferation, our observations indicate that the activation of p38 and p54 MAP kinase is not required for IL-2-driven cell cycle progression. This conclusion is supported by studies using the tetravalent guanylylhydrazone CNI-1493 (41), which inhibited IL-2-induced p38 and p54 MAP kinase activation but had no effect on IL-2-driven proliferation of either BA/F3 cells or T cells. Our data therefore suggest that the previously observed inhibition of IL-2-induced proliferation by the inhibitor SB203580 is likely to be due to its action on a target(s) other than p38 MAP kinase, which is required for cell cycle progression.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents**—The murine IL-2-dependent T cell line CT6 (kindly provided by Genentech, S. San Francisco, CA) was maintained in guanylate-supplemented RPMI 1640 (Biowhittaker, Ver- viers, Belgium) containing 5% fetal bovine serum (Sigma, Poole, Dorset, UK), 1 unit/ml penicillin/streptomycin (BioWhittaker), and 50 \( \mu \)g/ml G418 (Life Technologies Ltd., and PD098059 was from New England Biolabs (Hitchin, Herts, UK). Rabbit antibodies to p54 (SARK10) and p38 (SARK7) MAP kinase were provided by Prof. J Saklatvala (Kennedy Institute of Rheumatology, London) (44). Antibody to p42 MAP kinase/extracellular signal-regulated kinase 2 was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies to phosphorylated p42, p38, and p54 MAP kinases were from New England Biolabs. Antibodies to She and Grb2 and the anti-phosphotyrosine antibody P2-20 were from Transduction Labs (Lexington, KY). 4G10 anti-phosphotyrosine antibody was from Upstate Biotechnology, Inc. (Lake Placid, NY). The cDNA for histidine-tagged MAPK-activated protein kinase 2 was the generous gift of Prof. M. Gaestel (Max Delbruck Center for Molecular Medicine, Berlin, Germany). 

**Immunoprecipitation**—p38 MAP kinase and c-Jun N-terminal kinase/stress-activated protein kinase were immunoprecipitated from cleared cell lysates as described previously (40). She immunoprecipitations were performed as described previously (33).

**In Vitro Kinase Assays**—In vitro kinase assays for p38 and p54 MAP kinase activation were performed on precipitated immune complexes. p54 MAP kinase assays were performed as described previously using glutathione S-transferase-activating transcription factor 2 (GST-ATF2) as a substrate (40). In vitro kinase assays for p38 MAP kinase were performed using His_{6}-MAPK-activated protein kinase 2 as a substrate. Immunoprecipitates were incubated with 30 \( \mu \)l of kinase assay buffer (25 \text{mM HEPES}, pH 7.5, 25 \text{mM MgCl}_2, 25 \text{mM }\beta\text{-glycerophosphate}) containing 50 \( \mu \text{g/ml recombinant His}_{6}\text{-MAPK-activated protein kinase p38, 30 }\mu\text{M ATP, and 0.5 }\mu\text{Ci of }[^{32}\text{P}]\text{ATP (Amersham International, Little Chalfont, Buckinghamshire, UK) for 25 min at room temperature. Reactions were terminated by the addition of gel sample buffer and boiling for 5 min. All substrates were separated by SDS-polyacryl- amide gel electrophoresis. Gels were dried, and phosphorylated sub- strates were visualized using a Fuji FLA-2000 Imager (Raytek Scientific Ltd, Sheffield, UK) and by autoradiography at \( \sim 70^\circ\text{C} \).**

**Western Immunoblotting**—p42/44 MAP kinase (extracellular signal-regulated kinase 1/2) and phosphotyrosine Western blotting was performed as described previously (34). Western blotting for She, Grb2, and phosphorylated forms of p42/44, p38, and p54 MAP kinases was performed according to the antibody manufacturer’s instructions.

**RESULTS**

**Both the Serine-rich and the Acidic Domains of the IL-2R{\beta} Chain Are Required for MAP Kinase Activation by IL-2 in BA/F3 Cells**—IL-3-dependent BA/F3 cells normally express both the \( \alpha \)- and the \( \gamma \)-chains, but not the \( \beta \)-chain, of the IL-2R. When transfected with the IL-2R{\beta} subunit, they become responsive to IL-2 (4). We have used BA/F3 cell lines expressing either the wild type IL-2R{\beta} chain (F7) or mutant forms of the IL-2R{\beta} chain, lacking either the serine-rich region (S25) or the acidic region (A15) (Fig. 4A), to examine the role of the IL-2R{\beta} chain subdomains in the activation of p38 and p54 MAP kinase. The serine-rich and the acidic domains of the \( \beta \)-chain are essential for p42/44 MAP kinase activation (30); this is confirmed in Fig. 2A by detection of the phosphorylated kinase by Western blotting using a phosphospecific p42/44 MAP kinase antibody. Using immunokinease assays (for p38 and p54; Fig. 2B) or Western blotting for the phosphorylated, activated form (p38 only, Fig. 2A) to measure p38 and p54 MAP kinase activation, we have shown that these kinases are stimulated by IL-2 in F7 cells (Fig. 2, A and B). However, IL-2 was unable to activate either p38 or p54 MAP kinase in the absence of the serine-rich (S25) or acidic (A15) regions, indicating that both regions are required for kinase activation. IL-3 activated p42, p38, and p54 MAP kinases in all three cell lines and is included as a positive control. Kinetic experiments examining p38 and p54 MAP kinase activation over a 2-h period have established that the activation of these kinases by IL-2 is not simply re- duced in the S25 and A15 cell lines (data not shown).

**IL-2 Mediates Activation of p38 and p54 MAP Kinase Activation in BA/F3 Cells through Recruitment of She to the IL-2R{\beta} Chain Tyrosine Tyr^{355}**—This similarity in the \( \beta \)-chain subdomain requirements led us to examine whether, as for the activation of p42 MAP kinase, She is involved in the activation of p38 and p54 MAP kinases. A second panel of BA/F3 clones expressing mutant forms of the IL-2R{\beta} chain was used (Fig. 1B). In the cell line \( \Delta355 \), a truncation of the \( \beta \)-chain at amino acid 355 removes the proline-rich region and part of the acidic region to eliminate all of the cytoplasmic tyrosine residues except the Tyr^{358} residue required for She binding. IL-2 is nonetheless able to activate both p38 and p54 MAP kinases in...
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Fig. 1. Schematic diagram of the IL-2Rβ chain mutants expressed in the stably transfected BA/F3 cell lines used in this study.

this cell line (Fig. 3A). However, in another BA/F3 cell line expressing a full-length IL-2Rβ chain with a point mutation of Tyr338 to phenylalanine (Y338F), IL-2 fails to activate p38 and p54 MAP kinases, as determined by assay kinase and phosphospecific Western blotting (Fig. 3A). Curiously, there is still a very slight residual activation of all three MAP kinases by IL-2 when the Y338F mutation is combined with the Δ355 truncation in the BA/F3 cell line Δ355:Y338F (Fig. 3A and B). An additional BA/F3 line, Δ325-Shc, bears a version of the IL-2Rβ chain in which the entire acidic and proline-rich regions, including all cytoplasmic tyrosines of IL-2Rβ, are replaced with a covalently tethered Shc molecule to specifically reconstitute Shc-mediated signals, as described previously (42). IL-2 promotes p38 and p54 MAP kinase activation in these Δ325-Shc cells, suggesting that Shc may mediate the activation of p38 and p54 MAP kinases by IL-2 (Fig. 3A). However, the presence of the receptor fusion protein appears to compromise the ability of IL-3 to activate p38 and p54 MAP kinases. This may indicate that the activation of these kinases by IL-3 also occurs through Shc if the IL-2Rβ/ Shc fusion protein is somehow inhibiting a functional interaction between endogenous Shc and the IL-3 receptor. Fig. 3B shows p42 MAP kinase Western blots, confirming the role of the IL-2Rβ chain tyrosine Tyr338 and Shc in p42 MAP kinase phosphorylation and activation.

IL-2-driven Proliferation of BA/F3 Cells Is Suppressed by the p38 MAP Kinase Inhibitor SB203580—The observation that the IL-2Rβ chain acidic region is dispensable for IL-2-driven proliferation but that both the acidic and the serine-rich regions must be present for p38 and p54 MAP kinase activation in BA/F3 cells indicates that neither kinase is required for proliferation. This conflicts with our previous suggestion (40), based on studies using the p38 inhibitor SB203580, that p38 MAP kinase activation is required for IL-2-driven T cell proliferation. We therefore examined whether proliferation of the IL-2-responsive BA/F3 cell lines was also sensitive to this drug. Of the cell lines used, only two did not proliferate in response to IL-2: the S25 cell line, lacking the entire serine-rich region (4), and the Δ355:Y338F cell line, in which the IL-2Rβ chain lacks all three of the tyrosine residues of which at least one is required for proliferation (5, 42). Treatment of the cell line F7, A15, or Y338F with SB203580 (0.1–30 μM) resulted in the inhibition of IL-2-driven DNA synthesis with an IC50 of ~2–6 μM (Fig. 4). Proliferation of the other cell lines was similarly inhibited in each case (data not shown). The IC50 of SB203580 on IL-2-driven proliferation observed in these cell lines was comparable with that observed previously in T cells (40). However, since no activation of MAP kinases could be detected in either the A15 or the Y338F cells, in these cell lines the effect of SB203580 on proliferation could not be due to the inhibition of p38 MAP kinase and must instead reflect an effect of SB203580 on an unknown target.

CNI-1493 Inhibits the IL-2 Activation of p38 MAP and p54 MAP Kinases but Has No Effect on IL-2-driven Proliferation in the Murine T Cell Line CT6, BA/F3 Cell Lines, or Primary T Cells—It is possible that the discrepancy between the data presented here and our previous studies is due to cell type differences in IL-2 signaling between the CT6 T cell line used previously and the pro-B, BA/F3 cell lines used here. In order to address this possibility, we have made use of a second synthetic inhibitor. CNI-1493, a tetravalent guanylhydrazone compound, has been shown to inhibit the production of proinflammatory cytokines by monocyte/macrophage cells in response to lipopolysaccharide (46). The inhibition of proinflammatory cytokine production appears to operate at a post-transcriptional level (47), and there is some preliminary evidence to suggest that CNI-1493 is able to inhibit activation of p38 MAP kinase (48). We have shown that CNI-1493 does inhibit the lipopolysaccharide-induced phosphorylation and activation of p38 MAP kinase in both human monocytes and the murine macrophage cell line RAW 264.7, and in addition we have observed that both p42 MAP kinase/ extracellular signal-regulated kinase 2 and p54 MAP kinase are similarly inhibited.2 In CT6 cells, 1 μM CNI-1493 was found to completely ablate both p54 and p38 MAP kinase activation but to have no effect on p42/extracellular signal-regulated kinase phosphorylation induced by IL-2 (Fig. 5). The ability of CNI-1493 to inhibit p54 and p38 MAP kinase activation provided us with a tool with which to reassess the role of the MAP kinases in proliferative responses of T cells. CNI-1493 had no effect on IL-2-driven proliferation of CT6 cells or activated primary human T cells at doses that inhibit p38 and p54 MAP kinase activation in CT6 cells (Figs. 5 and 6A). Combinations of CNI-1493 and the MAP kinase kinase 1/2 inhibitor PD098059 (49), which inhibited the activation of all three MAP kinases, also failed to suppress the proliferation of CT6 cells, indicating that in T cells there is no redundant usage of MAP kinases in IL-2-driven proliferation (Fig. 7). This observation confirms the data obtained from the A15 and Y338F BA/F3 cell lines, which proliferate normally in response to IL-2 but in which no IL-2-stimulated MAP kinase activation was detected. The addition of CNI-1493 over 5 days failed to prevent cell propagation, suggesting that p38 and p54 MAP kinases are also not required for long term cell survival (data not shown). Similar effects on MAP kinase activation of CNI-1493 were observed in BA/F3 F7 cells (Fig. 5), although at 5–10 μM CNI-1493, inhibition of p42 MAP kinase phosphorylation was observed, an effect that was not seen in CT6 cells (data not shown). CNI-1493 was seen to have no effect on IL-2-driven

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This text is extracted from a scientific publication discussing the role of specific subdomains in the IL-2Rβ chain and the effect of inhibitors on MAP kinase activation in different cell lines. The text describes experiments using various cell lines and inhibitors to probe the role of MAP kinases in T cell proliferation and cytokine production. The study highlights the importance of specific subdomains in the IL-2Rβ chain and the differential effects of inhibitors on MAP kinase activation in different cell types and contexts. The text is rich with experimental details, including the use of Schematic diagrams and Western blots to illustrate the effects of mutations and inhibitors on MAP kinase activation and cell proliferation.
BA/F3 cell proliferation, even in the Δ355 cell line, in which Tyr338 is the only intracellular tyrosine residue present and proliferation is Tyr338-dependent (Fig. 6B). This observation indicates that these kinases are not involved in proliferative signaling even as a functionally redundant pathway only required in the absence of proliferative signals from the other intracellular tyrosines Tyr392 and Tyr510.

CNI-1493 Blocks p38 and p54 MAP Kinase Activation at a Point Distal to Shc and Grb2—Because receptor mutagenesis studies indicated a role for Shc in p38 and p54 MAP kinase activation, we examined the effects of CNI-1493 on IL-2-stimulated Shc phosphorylation in both BA/F3 cells and CT6 cells
by immunoprecipitation of Shc and phosphotyrosine Western blotting. CNI-1493 has no effect on IL-2-induced Shc tyrosine phosphorylation in either cell type, and subsequent Western blotting for Grb2 reveals that the interaction between Shc and Grb2 is also unaffected by this compound (Fig. 8). This observation suggests that the target of this drug must lie down-stream of Shc and Grb2 and is consistent with the fact that CNI-1493 has no effect on p42/44 MAP kinase activation. However, in the D325-Shc cell line the inhibitory effects of CNI-1493 on kinase activation are lost (data not shown). This result may indicate that the receptor-bound Shc can provide an alternative to the CNI-1493-sensitive component of the p38/p54 MAP kinase activation pathway. Alternatively, the loss of sensitivity to CNI-1493 may simply be a result of the overexpression of IL-2Rb-Shc fusion, a hypothesis that is supported by the observation that the inhibition of p42 MAP kinase phosphorylation by high doses of CNI-1493 is also lost in this cell line despite the fact that p42 MAP kinase phosphorylation is Shc-dependent.

**DISCUSSION**

This study has shown that the IL-2 activation of p38 and p54 MAP kinases, like that of p42 MAP kinase, requires both the acidic and the serine-rich regions of the IL-2Rb chain. In particular, activation of these kinases requires the IL-2Rb chain Tyr338 and appears to involve the recruitment of Shc. Since it has been well established that the acidic region of the IL-2Rb chain is not required for IL-2-induced proliferation, our results indicate that p38 MAP kinase and p54 MAP kinase are dispen-
IL-2 (20 ng/ml) or IL-3 (5% WEHI supernatant) as indicated. Cell treated for 1 h with CNI-1493 as shown and stimulated for 10 min with BA/F3 cells with an IC\textsubscript{50} of around 4 \textmu M, similar to that observed in IL-2-stimulated T cells, regardless of whether p38 MAP kinase was activated upon IL-2 stimulation. This observation raises doubts as to the specificity of the inhibitor in the cells used here. We have shown previously in CT6 cells that SB203580 is capable of inhibiting the IL-2 activation of MAPK-activated protein kinase 2, the immediate downstream kinase substrate of p38 MAP kinase, with an IC\textsubscript{50} of 0.3–0.5 \textmu M. In contrast, the inhibitory effects of SB203580 on the IL-2-driven proliferation of these cells are seen at drug concentrations approximately 1 order of magnitude higher. We originally suggested that this discrepancy may be due to the differences in the nature of the proliferation and kinase assays. Two very recent papers have demonstrated that at higher concentrations SB203580 directly inhibits the activity of the p54 MAP kinase isoform c-Jun N-terminal kinase 2 (50, 51). However, as we have shown here, p54 MAP kinase activation is not a requirement for BA/F3 cell proliferation; therefore, the inhibition of this kinase is unlikely to be responsible for the effects of SB203580 observed. Therefore, it must be concluded that at higher drug doses other target(s) are affected and that it is through its effect on this secondary target that the antiproliferative effects of SB203580 are mediated. This conclusion has wider implications. Since the discovery of SB203580, many cellular functions have been ascribed to p38 MAP kinase purely on the basis of their inhibition by SB203580 at doses well in excess of 1 \textmu M. It should now be considered that unless the effects of SB203580 on a particular physiological response can be seen at the doses at which p38 MAP kinase signaling is inhibited (0.1–0.5 \textmu M), no definite conclusions can be drawn to the involvement of p38 MAP kinase in mediating the effect.

In order to compare the mechanism of activation of MAP kinases and their role in proliferation in pro-B cells with that in T cells, we have made use of a tetravalent guanylhydrazone inhibitor of p38 MAP kinase phosphorylation, CNI-1493 (48). CNI-1493 inhibits the production of proinflammatory cytokines by monocytes/macrophages but has no effect on cDC3/cDC28-induced cytokine production in T cells (52). We have demonstrated that not only is this compound capable of inhibiting both the phosphorylation and activation of p38 MAP kinase induced by lipopolysaccharide in monocytes and macrophages but that it can also inhibit the activation of p54 and p42 MAP kinases by lipopolysaccharide in these cells as well (data not shown). In this study, we have demonstrated that in CT6 and BA/F3 cells the IL-2 activation of p38 and p54 MAP kinases is inhibited by CNI-1493. In contrast, p42/44 MAP kinase activation is not inhibited by the same doses of CNI-1493, although we have observed inhibition of p42 MAP kinase activation in BA/F3 cells with higher concentrations of the drug (5–10 \textmu M). We have shown that CNI-1493, unlike SB203580, has no effect on proliferation in either CT6 cells, primary human T cells or in the IL-2-responsive BA/F3 cell lines, confirming that neither p38 nor p54 MAP kinase activation is essential for either T cell or BA/F3 cell proliferation. The actual function of these kinases in these cell types and the significance of their activation in IL-2 signaling remain unknown and are not addressed in this study. However, CNI-1493 has been used successfully to inhibit the toxic side effects of IL-2 in anti-tumor therapy, and it is suggested that this may be the result of its ability to inhibit tumor necrosis factor and NO production (53). An implication of our results is that CNI-1493 will not prevent IL-2-driven tumor necrosis factor and NO production (53). An implication of our results is that CNI-1493 will not prevent IL-2-driven expansion of T cell clones with anti-tumor activity while simultaneously preventing the release of toxic macrophage products.

The effects of CNI-1493 on MAP kinase activation and studies with receptor mutants raise a number of interesting questions about the architecture of the upstream signaling pathways responsible for MAP kinase activation by IL-2. The requirement for Tyr\textsuperscript{388} the Shc binding site, for the activation of p38 and p54, as well as p42, MAP kinases indicates that Shc may be a common origin for signaling to all three. A role for Shc in the activation of p38 and p54 MAP kinases is supported by the fact that the expression of an IL-2R\textbeta-Shc fusion protein in BA/F3 cells was able to compensate for the loss of the Tyr\textsuperscript{388} and restore the IL-2-induced activation of p38 and p54 MAP kinases.

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**Fig. 8.** CNI-1493 does not inhibit IL-2-induced Shc phosphorylation or Grb2 recruitment. CT6 (a) and BA/F3 F7 (b) cells were treated for 1 h with CNI-1493 as shown and stimulated for 10 min with IL-2 (20 ng/ml) or IL-3 (5% WEHI supernatant) as indicated. Cell lysates were incubated with an anti-Shc antibody or a nonimmune control antibody (NF) and protein G-Sepharose for 3 h at 4°C. Immuno precipitates were washed (lysis and wash buffers used are described in Ref. 33) and separated by SDS-polyacrylamide gel electrophoresis. Western blotting for phosphotyrosine (upper panels), Grb2 (center panels), and Shc (lower panels) was performed as described under “Experimental Procedures.”
kinases. The fact that CN1-1493 is able to discriminate between p42 and p38/54 MAP kinases indicates that there must be a bifurcation of the activation pathways of these kinases at a distinct point to Shc itself, with the target of CN1-1493 lying downstream of Shc on the p38/p45 MAP kinase arm of the activation pathway. From Shc, the activation of p42 MAP kinase follows an established pathway requiring the formation of a complex of Grb2 and Sos with phosphorylated Shc (31–33) and the subsequent recruitment of p38 and p54 MAP kinases by IL-2 in BA/F3 cells, like that of p42 MAP kinase, requires the presence of both the acidic and the basic domains of Shc. It is also shown that the activation of these kinases is not dependent on the presence of Tyr338 and the recruitment of Shc is not involved in the activation of p38 and p54 MAP kinases indicates that there must be a subsequent recruitment of Grb2 in either CT6 or BA/F3 cells is consistent with its lack of effect on p42 MAP kinase activation.

Although we have demonstrated that Tyr338 and Shc recruitment are required for p38 and p54 MAP kinase activation in BA/F3 cells, whether this is also the case in T cells is not known. We have shown previously that another T cell mitogen, IL-7, is capable of activating p38 and p54 MAP kinases in T cells (40) and have evidence to suggest that IL-4 is also able to activate these kinases in CT6 cells. However, neither IL-7 nor IL-4 is able to activate p42/44 MAP kinases, and no phosphorylation of Shc can be detected upon stimulation with either of these cytokines (34). This suggests that in T cells either the recruitment of Shc is not involved in the activation of p38 and p54 MAP kinases or that the activation of these kinases in the CT6 cell line proceeds through different pathways depending on the mitogen used. Transfection of granulocyte-macrophage colony-stimulating factor-IL-2Rβb subunit chimeras into CT6 cells or CTLL cells as described recently (42) will allow this question to be addressed.

In summary, we have shown that the activation of p38 and p54 MAP kinases by IL-2 in BA/F3 cells, like that of p42/44 MAP kinase, requires the presence of both the acidic and the serine-rich region of the IL-2Rβb chain and, in particular, is dependent on the presence of Tyr338 and the recruitment of Shc. It is also shown that the activation of these kinases is not required for IL-2-driven proliferation in either T cells or BA/F3 cells. Our data suggest the existence of a non-MAP kinase target of Shc. It is also shown that the activation of these kinases is not dependent on the presence of Tyr338 and the recruitment of Shc is not involved in the activation of p38 and p54 MAP kinases or that the activation of these kinases in the CT6 cell line proceeds through different pathways depending on the mitogen used. Transfection of granulocyte-macrophage colony-stimulating factor-IL-2Rβb subunit chimeras into CT6 cells or CTLL cells as described recently (42) will allow this question to be addressed.

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