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Mercuric Chloride Stimulates Distinct Signal Transduction Pathway for DNA Synthesis in a T-Cell Line, CTLL-2

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Abstract Exposure of an interleukin-2 (IL-2)-dependent murine T-cell line (CTLL-2) to mercuric chloride in in vitro culture induced a low but definite level of DNA synthesis in the absence of exogenous IL-2, and further enhanced the IL-2-induced DNA synthesis. Addition of anti-IL-2 or anti-IL-4 antibody to the culture, which neutralized all of the IL-2 or IL-4 activity, respectively, never inhibited the mercuric chloride–mediated DNA synthesis. Correspondingly, no detectable level of IL-2, IL-4, and IL-15 mRNA was found in mercuric chloride–treated CTLL-2 cells in our test condition. Stimulation of CTLL-2 cells with IL-2 induced phosphorylation on extracellular signal-regulated kinases more intensively than on c-Jun NH2-terminal kinases (JNKs), and provoked tyrosine phosphorylation of Janus kinases (JAKs) and signal transducers and activators of transcription (STATs). In contrast, by mercuric chloride stimulation, JNKs and c-Jun were preferentially phosphorylated, but no detectable level of phosphorylation was induced on JAKs and STATs. These findings provided a possibility that mercuric chloride promoted lymphocyte proliferation through a JNK-linked signal cascade in CTLL-2 cells, which differs from that triggered by IL-2. J. Cell. Biochem. 78:500–508, 2000.

Key words: mercuric chloride; signal transduction; IL-2-dependent T lymphocyte line; IL-2.

In vivo challenge of heavy metal salts such as mercuric chloride has been shown to induce autoimmune diseases, represented by increased serum immunoglobulin, autoantibody, immune complex deposit, and lymphocyte proliferation [Pelletier et al., 1988; Goldman et al., 1991]. Mercuric chloride has been also reported to be a potent stimulator of both T cells and B cells in vitro for proliferative response [Nakashima et al., 1994; Jiang and Möller, 1995; Hu et al., 1997]. Previously, we showed that exposure of murine T lymphocytes to mercuric chloride activated protein tyrosine kinase $p56^{lck}$. The cascade of the signal transduction for the mercuric chloride–mediated induction of cell proliferation remains, however, unknown.

Signals for T-cell proliferation are usually delivered in two steps: first, through antigen-mediated activation of T-cell receptors (TCR) for cytokine production and cytokine receptor expression, and secondly through cytokine receptor activation by cytokine for DNA synthesis. The former step has been shown to involve activation of nonreceptor protein tyrosine kinases such as $p56^{lck}$ and ZAP70, leading to subsequent activation of the Ras-Raf- extracellular signal-regulated kinases (ERKs) signaling cascade [Ettehadieh et al., 1992; Whitehurst and Geppert, 1996]. The latter step, the signal transduction pathway under cytokine (interleukin-2; IL-2) receptors, has also been investigated extensively, and the existence of several different pathways has been reported [Taniguchi and Minami, 1993; Karnitz and Abraham, 1996]. One such pathway is the Janus kinases–signal transducers and activators

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of transcription (JAK-STAT) cascade, which is widely used in the signal transduction process under most cytokine receptors [Ihle, 1995; Ihle et al., 1998]. The mitogen-activated protein kinase (MAPK) superfamily involves stress-activated protein kinases SAPK/c-Jun NH2-terminal kinases (JNKs) in addition to ERKs, and the SAPK/JNK signal transduction pathway has been recently shown to be activated in response to oxidative stresses for phosphorylating and activating c-Jun as a component of transcription factor AP-1 [Whitmarsh and Davis, 1996; Sanchez et al., 1994]. It is not known, however, whether the stress-linked JNK-c-Jun signal pathway would partially replace the TCR/IL-2-mediated classic pathway for DNA synthesis.

Although it has been observed that lymphocytes respond to mercuric chloride, leading to cellular activation and proliferation through production of some cell growth-promoting cytokines such as IL-2 [Nakashima et al., 1994; Jiang and Möller, 1995; Hu et al., 1997], the study leaves a major question about whether the proliferative response is also a direct effect of the signal delivered by mercuric chloride, which may be more important for disorders. In this study, we used IL-2-dependent T-cell lines, to elucidate a direct effect of mercuric chloride on a single population of lymphocytes. We compared mercuric chloride–mediated signal transduction pathways with IL-2-mediated pathways in a T-cell line, CTLL-2, and found that the mercury-stimulated signal pathway for T-cell proliferation, which preferentially involved JNK/c-Jun but not JAKs and STATs, was distinct from the IL-2-stimulated one.

MATERIALS AND METHODS

Antibodies

Anti-IL-2 and anti-IL-4 antibodies were produced by Upstate Biotechnology (Lake Placid, NY) and Genzyme (Cambridge, MA), respectively, which specifically bind and neutralize respective cytokines. Rabbit polyclonal anti-phosphotyrosine antibody was purchased from Transduction Laboratories (Lexington, KY), and monoclonal anti-phosphotyrosine antibody and rabbit polyclonal anti-phosphospecific tyrosine 694-phosphorylated STAT5 and anti-STAT5 antibody were obtained from Upstate Biotechnology. Rabbit polyclonal antibodies specific for JAK1, JAK3, and JNK were obtained from Santa Cruz Biotechnology, (Santa Cruz, California). Rabbit polyclonal anti-STAT1, STAT3, ERK and c-Jun antibodies were obtained from New England Biolabs, (Beverly, MA). Anti-phosphospecific threonine 202/tyrosine 204-phosphorylated ERK1/ERK2, threonine 183/tyrosine 185-phosphorylated JNK-1/JNK-2, serine 73-phosphorylated c-Jun, tyrosine 701-phosphorylated STAT1 and tyrosine 705-phosphorylated STAT3 rabbit antibodies, which were prepared by immunizing rabbits with peptides containing phosphoamino acids, were also from New England Biolabs. Specificities of the antibodies were confirmed by positive reaction with the phosphorylated standard proteins (supplied by the manufacturer) and negative reaction with unphosphorylated proteins.

Cell Culture

The CTLL-2 cells were maintained and grown at 37°C, 5% CO_2 in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 μM 2-mercaptoethanol and 10% IL-2-containing medium, which was from the culture supernatant of murine spleen cells stimulated with Concanavalin A (Con A). For experiments requiring growth factor–deprived cells, cells were cultured for 4 h in IL-2-free medium (RPMI 1640 medium containing 100 μg/ml bovine serum albumin and 50 μM 2-mercaptoethanol) before restimulation with human recombinant IL-2 or mercuric chloride ($HgCl_2$).

Proliferative Assay and Flow Cytometric Analysis

CTLL-2 cells were seeded in 96-well flat-bottom culture plates at 2×10^4 cells per well, and cultured in 200 μl of culture medium containing indicative concentration of mercuric chloride or IL-2. At 12 h before harvest, ^{3}H-thymidine (37 kBq per well, Amersham-Japan Ltd., Tokyo, Japan) was added. The cells were then harvested to filter paper, and the radioactivities were determined by liquid scintillation counting as described [Ohkusu et al., 1997]. To analyze cell cycle, cells were washed and suspended in propidium iodide solution (50 μg/ml in 0.3% sodium citrate with 0.01% Triton X-100) and incubated for 30 min on ice. Cells were analyzed using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

Reverse Transcriptase–Polymerase Chain Reaction Analysis

RNA was isolated using the acid guanidinium-phenol-chloroform) method. Total RNA from 6×10^6 CTLL-2 cells was used for reverse transcriptase (RT)-mediated synthesis of cDNA using the SuperScript Preamplification System (Life Technologies, Rockville, MD). Polymerase chain reaction (PCR) was carried out in a volume of 50 μl containing 2 μl of the reverse transcription mixture. PCR for the amplification of cytokine gene products was started with a denaturing step for 3 min at 94°C, followed by 35 cycles of three PCR steps of 60 s at 94°C, 60 s at 60°C (IL-2 and IL-4), or 50°C (IL-15) and 90 s at 72°C, and terminated with an extension prolongation for 5 min at 72°C. PCR cycles for β-actin were limited to 20 to avoid amplification saturation. The specific murine IL-2 (sense: 5′-TGATGGACCTACAGGAGCTCCTGAG-3′, antisense: 5′-GAGTCAAATCCAGAACATGCCGCAG-3′), IL-4 (sense: 5′-CGAAGAACACCACAAAGACAGTGAGCT-3′, antisense: 5′-GACTCATTCATCGTGCAGCTTATCG-3′) IL-15 (sense: 5′-GTGATGTTCACCCCAGTTGC-3′, antisense: 5′-TCACATTCTTTGCATCGAGA-3′) and β-actin specific primer used for PCR analysis have been described previously [Suzuki et al., 1997]. After the PCR amplification reaction, the PCR products were analyzed by ethidium bromide staining in 1.8% agarose gels using standard techniques.

Immunoprecipitation and Western Blot Analysis

Cells (2×10^7 cells per sample) were lysed in 1 ml of RIPA buffer [20 mM Tris (pH 7.4), 40 mM NaCl, 5 mM EDTA, 30 mM $Na_4P_2O_7$, 50 mM NaF, 1% Triton X-100, 0.1% bovine serum albumin, 1 mM sodium orthovanadate, 20 mM *p*-nitrophenyl phosphate, and 5 μg/ml each of aprotinin, leupeptin, and pepstatin, and 0.5 mM phenyl methyl sulphonyl fluoride]. The lysates were cleared of insoluble material by centrifugation, and phosphotyrosine-containing proteins were immunoprecipitated from them with a monoclonal anti-phosphotyrosine antibody and protein A–coupled Sepharose beads as described before [Akhand et al., 1997]. The immunoprecipitates were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS/PAGE), and immunoblotting was performed with anti-JAK1 and JAK3 antibodies. The cell lysates or proteins immunoprecipitated as described above were separated by SDS-PAGE on 8% gel, and transferred to a polyvinylidene difluoride microporous membrane (Millipore, Bedford, MA). The proteins on the membrane were probed with indicated antibodies. Immunoreactive proteins were visualized by enhanced chemiluminescence.

RESULTS

Effect of Mercuric Chloride on Proliferation of CTLL-2 Cells

We analyzed DNA synthesis in CTLL-2 cells that were treated with mercuric chloride. IL-2-deprived CTLL-2 cells were cultured with different concentrations of mercuric chloride overnight, and their levels of DNA synthesis were examined by ^{3}H-thymidine uptake. As shown in Figure 1A, ^{3}H-thymidine uptake was increased by adding 0.01–10 μM of mercuric chloride in the culture without exogenous IL-2, with a highest level in the culture added with 0.1–1 μM. Treatment of IL-2-deprived CTLL-2 cells with 5–40 μM of mercuric chloride for 120 min before overnight culture without addition of IL-2 also increased their ^{3}H-thymidine uptake. This synthesis was more evident as the concentration of mercuric chloride increased (Fig. 1B). These results showed that mercuric ions affected CTLL-2 to deliver some signal for DNA synthesis, even though this signal was less potent than that delivered by IL-2.

We next examined a potential action of mercuric chloride on CTLL-2 cells in the presence of IL-2. As shown in Figure 1C, addition of 0.01–1 μM of mercuric chloride to the culture of CTLL-2 cells in the presence of IL-2 further promoted the IL-2-mediated DNA synthesis. This result suggested that the mercuric chloride–provoked signal worked together with the IL-2-mediated one for promotion of DNA synthesis.

Flow cytometry profiles of nuclear DNA content were next examined. As shown in Figure 2, treatment of CTLL-2 with mercuric chloride promoted cell cycle progression from G0/G1 to G2/M phase (10.5% as compared with 6.4% in untreated control cells), although this movement of cell cycle is less evident than that induced by IL-2 (26.9% in G2/M phase).

Mercuric Chloride–Induced CTLL-2 Cell Proliferation Is Cytokine Independent

To determine the possibility that the mercuric ions acted to promote proliferation of

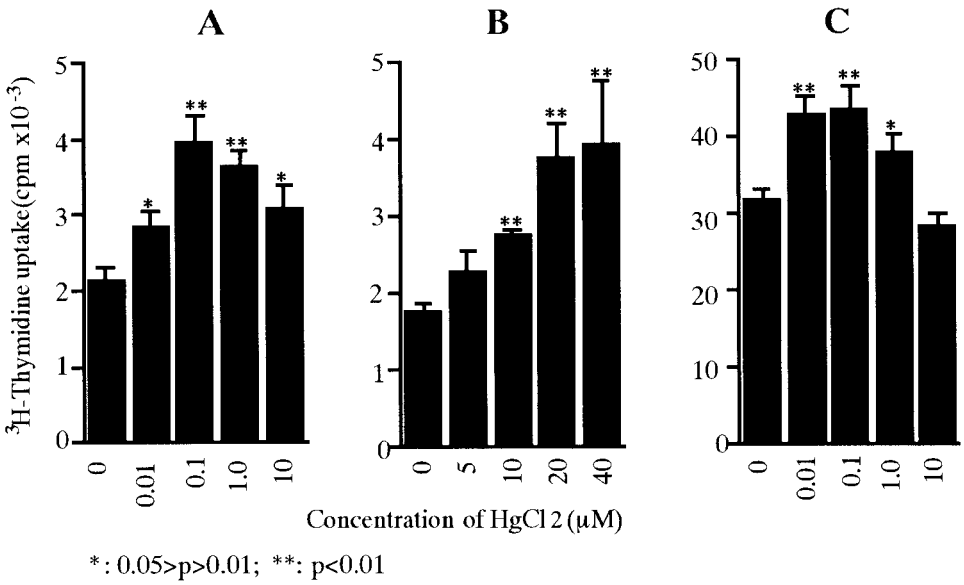

Fig. 1. Mercuric chloride induces proliferation of CTLL-2. Growth factor-deprived CTLL-2 cells (2×10^4 cells per well) were cultured with or without different concentrations of mercuric chloride ($HgCl_2$) for 24 h. At the last 12 h, ^{3}H-thymidine (37 kBq per well) was added, and radioactivity uptake was measured. **A:** Cells were cultured with mercuric chloride in the absence of IL-2. **B:** Cells were pretreated with mercuric chloride for 120 min. They were then washed three times with minimum essential medium, followed by culture in the absence of IL-2; **C:** Cells were cultured with mercuric chloride in the presence of exogenously added IL-2 (25 U/ml). Mean counts per minute (cpm) from triplicate cultures is shown as the representative of three separate experiments. Statistically significant data are indicated by asterisks (*$P < 0.05$, **$P < 0.01$, by Student's *t* test).

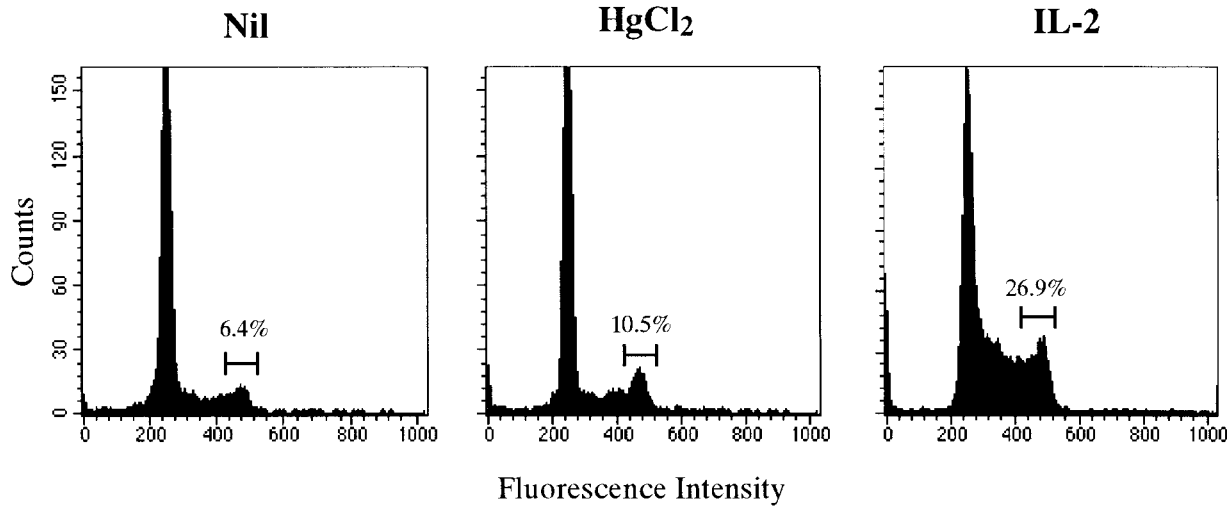

Fig. 2. Cell cycle profile of mercuric chloride–stimulated CTLL-2 cells. CTLL-2 cells were cultured with ($HgCl_2$) or without (Nil) 0.1 μM mercuric chloride for 24 h and their cell cycle profile was analyzed as described in Methods. The profile of IL-2-stimulated CTLL-2 cells (IL-2, 25 U/ml) is also demonstrated. Percentages of cells in G2/M phase (6.4%, 10.5%, and 26.9% for Nil, $HgCl_2$, and IL-2, respectively) are indicated in the histogram windows. Three separate experiments were carried out with results similar to the representative data shown here.

CTLL-2 cells through inducing cytokine production, we performed an anti-IL-2 antibody (Ab) blocking and anti-IL-4 Ab blocking test for CTLL-2 cells. As shown in Figure 3A, addition of anti-IL-2 Ab and IL-4 Ab, which completely repressed IL-2-induced and IL-4-induced proliferation, respectively, did not inhibit mercuric chloride–induced proliferation. We next measured IL-2, IL-4, and IL-15 expression in mercuric chloride–treated CTLL-2 cells by RT-

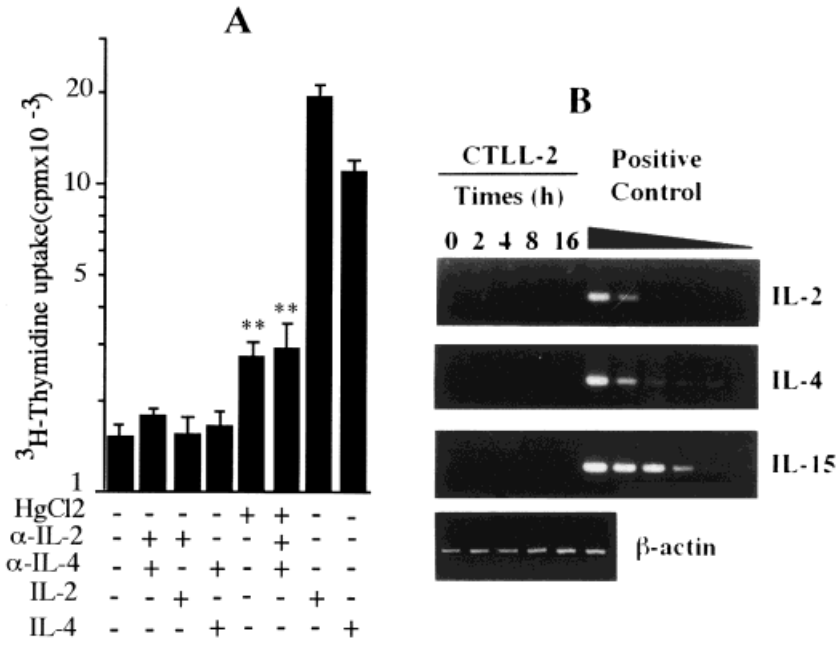

Fig. 3. Mercuric chloride–induced proliferation of IL-2-dependent cell lines is cytokine independent. **A:** Growth factor–deprived CTLL-2 cells were cultured with or without 1 μM mercuric chloride, 25 U/ml IL-2, and 50 U/ml IL-4, plus 1.0 μg/ml anti-IL-2 Ab, and 2.0 μg/ml anti-IL-4 Ab for 24 h, for analysis of ^{3}H-thymidine uptake. The cpm of control culture (addition of neither mercuric chloride nor IL-2) was 1,288 ± 92.1. Mean values of cpm from three independent experiments are presented. **Significantly different from control ($P < 0.01$ by Student's *t* test). **B:** CTLL-2 cells were stimulated for indicated hours with 1 μM mercuric chloride, and the gene expression of IL-2, IL-4, and IL-15 was measured by RT-PCR analysis (upper three panels). Splenic cells from C57BL/6 mice stimulated with Con A for 4 h were used as the positive control for IL-2 and IL-4 expression, and J774.A1 (a macrophage/monocyte cell line) stimulated with 5 μg/ml LPS for 6 h was used for the IL-15 expression control. These control samples were serially diluted as 1/1, 1/10, 1/100, 1/1,000, 1/10,000, 1/100,000 from left to right, respectively. The expression levels of β-actin are shown as a loading control.

PCR analysis. CTLL-2 cells do not express IL-2 at the detectable mRNA level by the stimulation with mercuric chloride up to 16 h (Fig. 3B, top panel). The expression of IL-4 and IL-15, which are possible proliferation-inducing factors for CTLL-2 cells, were also under undetectable levels by RT-PCR assay (Fig. 3B, second and third panels). The C57BL/6 mouse splenic cells stimulated with Con A were tested in parallel as the positive control for IL-2 and IL-4, and a macrophage/monocyte cell line stimulated with LPS was used as the control for IL-15 (Fig. 3B, right half of each panel). Serial dilution of these reverse-transcribed control samples revealed that the expression levels of IL-2, IL-4, and IL-15 from mercuric chloride–stimulated cells were less than 1/100, 1/10,000, and 1/100,000 of control cells, respectively. These results demonstrated that mercuric chloride could switch on a signal pathway for inducing proliferation of CTLL-2 cells, which was independent of IL-2, IL-4, and IL-15.

SAPK/JNKs and c-Jun Are Preferentially Phosphorylated by Mercuric Chloride–Stimulation

We conducted further experiments to determine whether the MAPK family kinase cascade was similarly activated by mercuric chloride and IL-2. CTLL-2 cells, which had been stimulated with IL-2 or mercuric chloride for 15–60 min, were subjected to Western blot analysis with anti-phosphospecific antibodies for demonstrating activated forms of ERKs, JNKs, and c-Jun that are phosphorylated at specific amino acid residues. The results are shown in Figure 4. On stimulation with IL-2 or mercuric chloride, a rapid induction of specific tyrosine phosphorylation was observed in p42ERK1 and p44ERK2. However, this increase continued for a shorter period after stimulation with mercuric chloride than after stimulation with IL-2 (Fig. 4A). We further observed that p54 SAPKβ (JNK2) but not p46 SAPKγ (JNK1) was phosphorylated by the stimulation with IL-2. Inter-

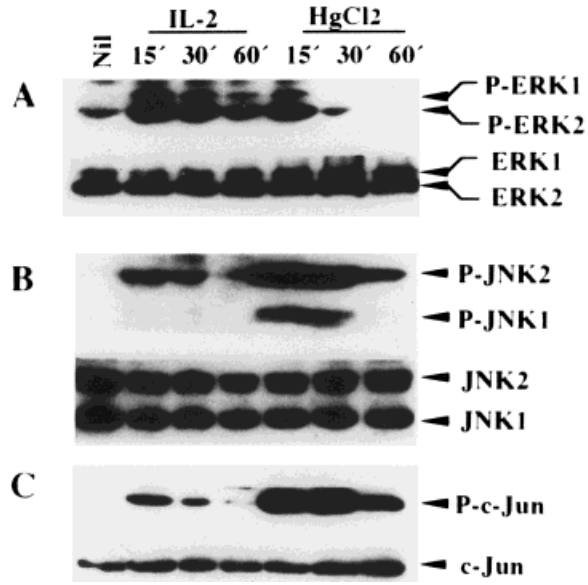

Fig. 4. Mercuric chloride and IL-2 differentially promote phosphorylation of ERKs, JNKs, and c-Jun. Growth factor–deprived CTLL-2 cells were treated for 15, 30, and 60 min with 100 U/ml IL-2 or 20 μM mercuric chloride and then lysed in sample buffer. The lysates were separated by SDS-PAGE and subjected to Western blot analysis with anti-phosphospecific phosphorylated ERK1/ERK2 [P-ERK1 and P-ERK2, (**A**)] JNK1/JNK2 [P-JNK1 and P-JNK2, (**B**)] and c-Jun [P-c-Jun, (**C**)] antibodies. Western blot analysis with anti-ERKs [ERK1 and ERK2, (A)], anti-JNKs [JNK1 and JNK2, (B)], and anti-c-Jun [c-Jun, (C)] antibodies are also demonstrated to show invariable protein levels of these elements among the samples.

estingly, stimulation with mercuric chloride was found to induce more extensive phosphorylation of both JNK2 and JNK1 than stimulation with IL-2 (Fig. 4B). Correspondingly, a more extensive and longer period of phosphorylation was induced on c-Jun by the former stimulation than by the latter (Fig. 4C). These observations suggested that whereas IL-2 mainly and moderately activated ERKs/c-Jun, mercuric chloride preferentially and extensively activated the JNK/c-Jun signal cascade in CTLL-2 cells.

Tyrosine Phosphorylation of JAKs and STATs Is Not Induced by Mercuric Chloride Stimulation

JAK and STAT proteins are known to play a critical role in IL-2-mediated gene expression regulation. We next asked whether theses proteins are involved in the mercuric chloride–mediated signal pathway. To assess the tyrosine phosphorylation level of JAK proteins, CTLL-2 cells stimulated with IL-2 or mercuric chloride for 10 min were subjected to immunoprecipitation with anti-phosphotyrosine antibody followed by immunoblot analysis with anti-JAK1 or anti-JAK3 antibody. As shown in Figure 5A, mercuric chloride–stimulated CTLL-2 cells showed no increase in tyrosine phosphorylation of either JAK1 or JAK3 protein in contrast to the marked increase in tyrosine phosphorylation of both JAK1 and JAK3 in IL-2-stimulated CTLL-2 cells.

The results of STATs phosphorylation profiles are shown in Figure 5B. The CTLL-2 cells treated with IL-2 or mercuric chloride for 15–60 min were subjected to SDS-PAGE followed by Western blot analysis with anti-phosphospecific phosphorylated STAT1, STAT3, and STAT5 antibodies. On stimulation with IL-2, tyrosine phosphorylation of STAT1, STAT3, and STAT5 was induced within 15 min, which gradually declined toward 60 min after IL-2 stimulation. In contrast, none of STAT1, STAT3, and STAT5 were tyrosine phosphorylated to any detectable level in response to mercuric chloride.

DISCUSSION

In this study, using a single cell population (CTLL-2), we have for the first time confirmed a direct effect of mercuric chloride on lymphocytes for promoting the DNA synthesis and cell cycle progression from G0/G1 to G2/M. This formally eliminated the possibility that mercuric chloride affects accessory cells rather than lymphocytes for the latter cell proliferation. It might be, however, that the production of IL-2 or other cytokines potentially induced by mercuric chloride in the lymphocyte was responsible for the mercuric chloride–induced lymphocyte proliferation. We provided evidence contradicting this view. First, anti-IL-2 Ab and anti-IL-4 Ab, which neutralized all the IL-2 and IL-4 activity, respectively, did not inhibit mercuric chloride–mediated CTLL-2 proliferation. Second, no expression of mRNA of IL-2, IL-4, and IL-15 gene was found in the mercuric chloride–treated CTLL-2 cells. Third, mercuric chloride delivered a signal that synergistically cooperates with the IL-2-mediated signal for a stronger proliferation response.

It is therefore concluded that mercuric chloride stimulation delivers a signal that is alternative to and possibly cooperative with the IL-2-mediated signal for DNA synthesis. Comparative studies of the signals delivered by

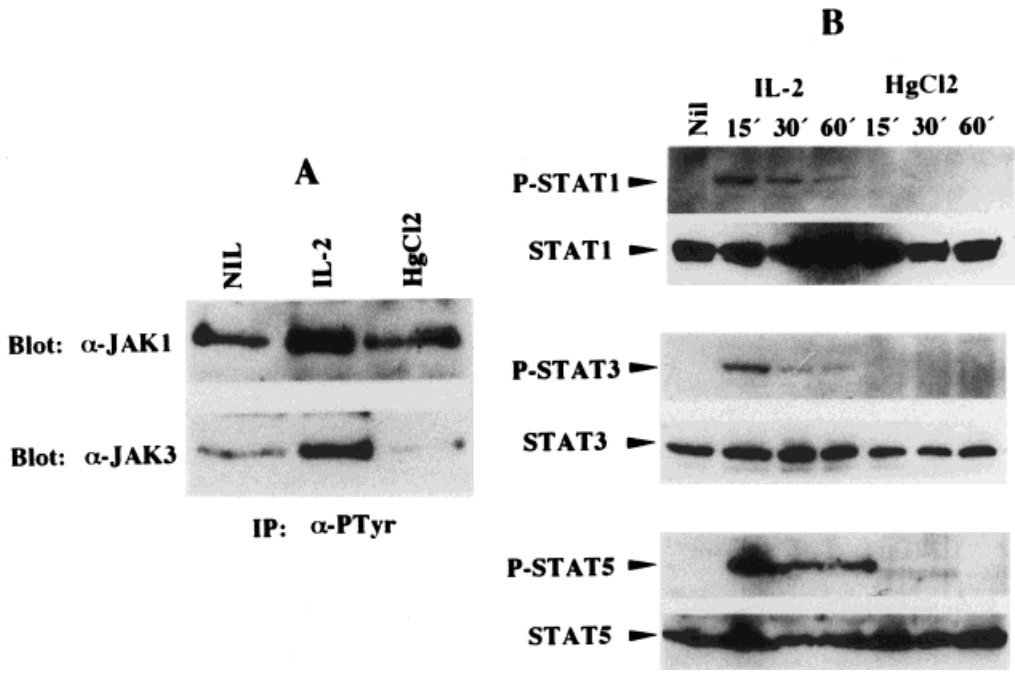

Fig. 5. IL-2 but not mercuric chloride induces tyrosine phosphorylation of JAKs and STATs. **A:** Cell lysates prepared from CTLL-2 cells stimulated with either mercuric chloride (20 μM) or IL-2 (100 U/ml) for 10 min were immunoprecipitated with anti-phosphotyrosine antibody. The precipitated samples were immunoblotted with anti-JAK1 or anti-JAK3 antibody. **B:** Growth factor–deprived CTLL-2 cells were stimulated with 100 U/ml IL-2 or 20 μM mercuric chloride for 15–60 min. These cells were lysed in sample buffer and immunoblotting was performed with anti-phosphospecific phosphorylated STAT1 (P-STAT1), STAT3 (P-STAT3), or STAT5 (P-STAT5) antibodies. The membranes were stripped of antibodies and then immunoblotted with anti-STAT1, STAT3, or STAT5 (STAT1, STAT3, STAT5, respectively) antibodies.

mercuric chloride and IL-2 evidenced different signal pathways. Stimulation of CTLL-2 cells with IL-2 triggered both MAPK family kinase-mediated and JAK-STAT-mediated signal pathways. The dominant elements activated by IL-2 in the MAPK family kinases were ERK1 and ERK2. This finding basically corresponds to the earlier report that, in addition to STATs, the activated ERK-dominant MAPK family kinase pathway plays an important role in IL-2-mediated signaling for proliferation in the CTLL-2 cells [Karnitz et al., 1995]. In contrast, stimulation with mercuric chloride predominantly provoked a JNK-mediated signal pathway, resulting in heavy phosphorylation of serine 73 of c-Jun, confirming that mercuric chloride dominantly activates the stress-responsive elements in the MAPK superfamily. JNK and ERK activities are associated with mitogenic signaling, proliferation [Robinson and Cobb, 1997], and activation [Hsueh and Lai, 1995; Su et al., 1994] of lymphocytes. Besides c-Jun, the JNK pathway also activates the AP-1 transcription factors including ATF-2 [Gupta et al., 1995], Elk-1 [Whitmarsh et al., 1995], and Sap-1a [Janknecht and Hunter, 1997]. Activities of AP-1 and NF-kB, which also interact with the JNK pathway [Meyer et al., 1996], are important for cell proliferation. Recently, it was shown that activation of JNK was a preferential effector pathway for the epidermal growth factor–induced [Bost et al., 1997] and IL-3-induced [Smith et al., 1997] growth and that activation of JNK correlated with a strong induction of proliferation in Epstein-Barr virus-immortalized primary human B cells [Kilger et al., 1998; Kieser et al., 1999], suggesting a role of JNK in proliferative response. In this report, we show that mercuric chloride activates both JNK and ERK pathways, which may contribute to promoting DNA synthesis in CTLL-2 cells, although ERKs activation continues a short period. Purified normal T cells do not show detectable levels of proliferation in response to mercuric chloride [Jiang and Möller, 1995; and our unpublished observation], indicating that mercuric chloride alone may not be sufficient to induce prolifer-

ative signals in normal resting T cells. CTLL-2, which is an already activated cell line, may have some advanced steps in the pathway for cell proliferation, being ready to respond to mercuric chloride.

Another important finding in the present work is the lack of involvement of tyrosine phosphorylation (activation) of JAKs and STATs in the mercuric chloride–mediated signal delivery in CTLL-2 cells. JAK-STAT signaling pathway was known as a pivotal responder for stimulation of cytokines. Recent studies have demonstrated that this pathway is also activated by many types of environmental stress. The JAK and STAT family, including JAK1, JAK2, Tyk2, STAT1, and STAT3, are activated in the different cell lines in response to hyperosmolarity [Gatsios et al., 1998]. Another report has shown that exposure to reactive oxygen species induces tyrosine phosphorylation of STAT1 and STAT3 [Simon et al., 1998]. Mercuric chloride may induce a different signaling pathway from these stresses. It was also reported that the JAK-STAT pathway was activated by numerous cytokines through their receptors, but signaling through the TCR stimulation did not activate this pathway [Beadling et al., 1994]. In our previous study, treatment with mercuric chloride delivered intracellular signals by co-crosslinking the cell surface molecules including Thy-1, CD3, CD4 and CD45 [Nakashima et al., 1994]. Thus, it is possible that TCR may be involved in the signal pathway induced by mercuric chloride, which does not require activation of JAKs and STATs.

In short, our results provided evidence that the mercuric chloride–induced signal pathway, which is distinct from but possibly cooperative with the IL-2-stimulated ERK/STAT-mediated pathways, works to promote proliferation of a non-IL-2-producing T-cell line. The potential role of this pathway in the dynamics of T-lymphocyte proliferation should be further investigated, particularly in relation to the mechanism of the mercuric chloride–induced lymphoproliferative disorders.

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