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INTERLEUKIN-2 (IL-2) INDUCES ERYTHROID DIFFERENTIATION AND TYROSINE PHOSPHORYLATION IN ELM-I-1 CELLS TRANSFECTED WITH A HUMAN IL-2 RECEPTOR β CHAIN cDNA

Kazunori IMADA[1,2], Mitsuru TSUDO[3], Tai-ichi KODAKA[2], Katsuhiko ITOH[2], Nobuyoshi ARIMA[1,2], Toshio HATTORI[1], Minoru OKUMA[2], and Takashi UCHIYAMA[1]

[1]Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaramachi, Sakyo, Kyoto 606, Japan

[2]First Division of Internal Medicine, Faculty of Medicine, Kyoto University, 54 Shogoin -Kawaramachi, Sakyo, Kyoto 606, Japan

[3]Department of Internal Medicine, Kyoto-Katsura Hospital, 17 Yamadahirao-cho, Nishikyo, Kyoto 615, Japan

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SUMMARY: The molecular mechanism of erythroid differentiation has been still ill-defined. In this study, we introduced a human interleukin-2 receptor (IL-2R) β chain cDNA into ELM-I-1 cells which differentiated into hemoglobin-positive cells in the presence of erythropoietin (Epo), and established the transformant which expressed IL-2R β chain. In this transformant, we revealed that IL-2 induced erythroid differentiation and the same pattern of tyrosine phosphorylation as Epo. These data suggest that tyrosine phosphorylation is involved in signal transduction pathway of erythroid differentiation. It is also implicated that the Epo and IL-2 receptor system share a common signal transduction pathway.

Erythropoietin (Epo) is a glycopeptide hormone that promotes the proliferation of erythroid progenitor cells and is required for their terminal differentiation (1). Although Epo has been well characterized and a cDNA encoding its surface receptor has been isolated (2), signal transduction pathway following Epo binding to its receptor has still remained uncertain. Recently, several reports have implicated that tyrosine phosphorylation is involved in signal transduction mediated by the Epo receptor (EpoR), when it induces proliferation (3-4). However, little is known on Epo-induced signal transduction, when it induces differentiation of the cells.

To investigate the signal transduction pathway of erythroid differentiation, we introduced a human IL-2R β chain cDNA into ELM-I-1 cells, because of a high degree of structural homology between the EpoR and IL-2R β chain (5), and examined biological response and tyrosine phosphorylation with Epo or IL-2 stimulation.

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MATERIALS AND METHODS

Cell line and monoclonal antibodies (MoAbs). ELM-I-1 is a murine erythroblastic leukemia cell line which differentiates into hemoglobin-producing cells in the presence of Epo (6-8). ELM-I-1 cells were maintained in alpha-MEM supplemented with 10% FCS (Gibco Laboratories). 2R-B is a murine anti-human IL-2R β chain MoAb (9). AMT-13 (10) and TM-β1(11) are rat anti-murine IL-2R α and β chain MoAbs, respectively.

Cytokines. Recombinant human Epo was a kind gift from Chugai Pharmaceutical Co., Tokyo, Japan. Recombinant human IL-2 was a kind gift from Takeda Chemical Industries Co., Osaka, Japan.

Transfection of human IL-2R β chain cDNA. ELM-I-1 cells were transfected with the expression plasmid, BCMGNeoβ1.9 (12), carrying the human IL-2R β chain cDNA by the electroporation method. In brief, ELM-I-1 cells ($5X10^6$) were transfected with 10 μg of BCMGNeoβ1.9 at 600 V and 25 μFD pulse by using an electric cell border (Bio-Rad). Transfected cells were selected by culturing in growth medium containing 0.75 mg/ml of G418 and then stable transformant which expressed IL-2R β chain, termed as ELM-I-1β1-18 was obtained by limiting dilution.

Immunoblotting with an antiphosphotyrosine antibody. Tyrosine phosphorylation was examined by immunoblotting with a polyclonal rabbit antiphosphotyrosine antibody (Zymed). Whole cell lysates were prepared as previously described (13). In brief, $1x10^6$ cells were stimulated with Epo or IL-2, rapidly pelleted by centrifugation for 10 seconds, and lysed in 50 μl of hot sodium dodecyl sulfate (SDS) lysis buffer containing 100 μM sodium vanadate and 25 mM dithiothreitol (DTT). Each sample was boiled for 5 minutes, and the DNA was sheared by several passages through a 26-gage needle. Whole cell lysates were loaded onto 7.5% SDS-polyacrylamide gels, electrophoresed, and transferred to a Immobilon- polyvinylidenedifluoride (PVDF) membrane (Millipore). Then, PVDF blots were incubated with 0.5 μg/ml of antiphosphotyrosine antibody for 1 hour, followed by washing and incubation with a horseradish peroxidase-conjugated anti-rabbit IgG antibody (1:4,000 dilution; Tago) for 1 hour. After washing, they were finally incubated with ECL solution (Amersham) for 1 minute and exposed on Hyper-ECL films (Amersham).

Cell differentiation assay. ELM-I-1 and ELM-I-1β1-18 cells were cultured in growth medium containing Epo or IL-2 at the initial cell density of $5X10^4$/ml. At the indicated times, hemoglobin-positive cells were counted after staining with 2,7-diaminofluorene (DAF) (14).

RESULTS AND DISCUSSION

Functional expression of IL-2R β chain. Flow cytometric analysis showed that ELM-I-1β1-18 cells expressed human IL-2R β chain, whereas their parental cells did not (Figure 1). Both ELM-I-1 and ELM-I-1β1-18 cells were not stained with AMT-13 and TM-β1 MoAbs confirming that they lacked the expression of murine IL-2R α and β chains (data not shown). IL-2

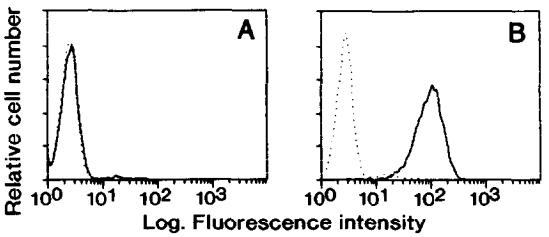

Figure 1. Flow cytometric analysis of the cell surface expression of human IL-2R β chain. ELM-I-1 (A) and ELM-I-1β1-18 (B) cells were stained with biotinylated 2R-B MoAb and PE-conjugated streptavidin (———). Background staining with PE-conjugated streptavidin is also shown (• • • • •).

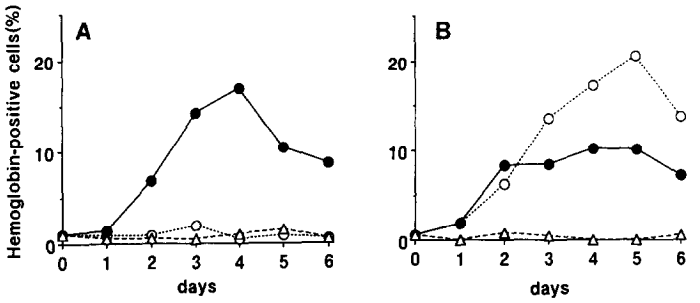

Figure 2. Kinetics of the appearance of hemoglobin-positive cell in the presence of Epo or IL-2. ELM-I-1 (A) and ELM-I-1β1-18 (B) cells were cultured in the growth medium containing 2U/ml of Epo (●—●) or 8 nM of IL-2 (○---○). Control cells were cultured in the absence of Epo and IL-2 (△--△). Hemoglobin-positive cells were counted after staining with DAF.

binding assay using [^{125}I] IL-2 revealed that ELM-I-1β1-18 cells expressed intermediate-affinity receptors (Kd=0.55 nM) and the number of expressed receptors was 720 (data not shown).

Effects of Epo and IL-2 on the transformant. ELM-I-1 cells were reported to differentiate into hemoglobin-producing cells in the presence of Epo (7). The hemoglobin-positive cells in ELM-I-1 and ELM-I-1β1-18 cells cultured with Epo appeared at day 2 (Figure 2). Epo induced the differentiation into hemoglobin-positive cells in both cells, whereas IL-2 induced the differentiation only in ELM-I-1β1-18 cells but not in ELM-I-1 cells (Figure 2). All these phenomena were observed in a concentration-dependent fashion (Figure 3). Cell proliferation assay revealed that neither Epo nor IL-2 affected the growth of ELM-I-1 and ELM-I-1β1-18 cells, when they were cultured in growth medium (data not shown).

Protein tyrosine phosphorylation in ELM-I-1 and ELM-I-1β1-18 cells. To analyze the early events of signal transduction, we examined tyrosine phosphorylation by immunoblotting. Exposure to Epo induced tyrosine phosphorylation of a protein with MW of 100 kD (designated pp100) in ELM-I-1 cells within 2 minutes (Figure 4). We next compared the tyrosine

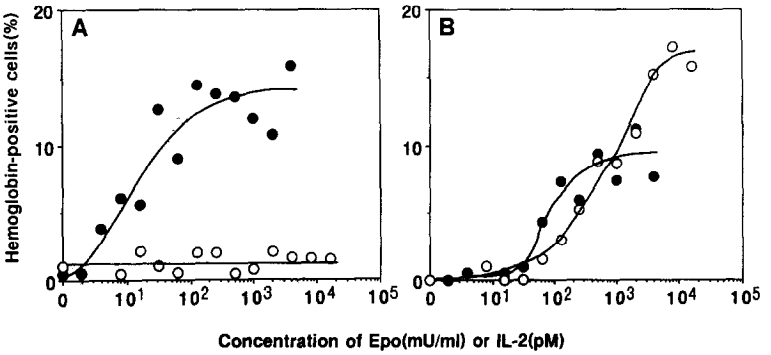

Figure 3. Dose response curve of Epo-induced (●—●) and IL-2-induced (○—○) appearance of hemoglobin-positive cells in ELM-I-1 (A) and ELM-I-1β1-18 (B) cells. Hemoglobin-positive cells were counted at day 4.

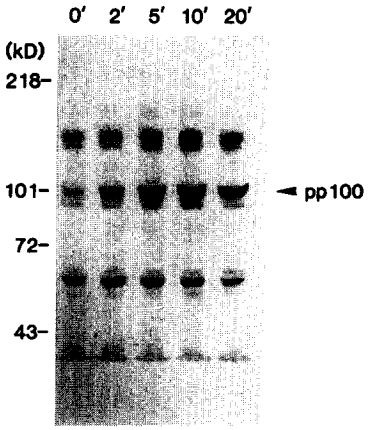

Figure 4. Time course study of Epo-induced protein tyrosine phosphorylation in ELM-I-1 cells. Cells were exposed to 2 U/ml of Epo for the indicated time and then were lysed and immunoblotted with an antiphosphotyrosine antibody as described in Materials and Methods.

phosphorylation events using ELM-I-1 and ELM-I-1β1-18 cells. Interestingly, Epo and IL-2 induced pp100 in ELM-I-1β1-18 cells, although only Epo induced tyrosine phosphorylation in ELM-I-1 cells (Figure 5).

In the present study, we showed that Epo and IL-2 induced the same differentiation and the same pattern of tyrosine phosphorylation in ELM-I-1β1-18 cells. Although EpoR and IL-2R have no recognizable intrinsic tyrosine kinase domain, our data suggest that tyrosine phosphorylation is early signaling events in erythroid differentiation. This system may be a useful model to analyze the molecular mechanism of erythroid differentiation.

On the other hand, in growth signaling, signal transduction pathway common to cytokine receptor systems has been predicted by various cDNA transfection experiments. For example, it has been reported that an IL-3-dependent line can be switched to an Epo-dependent state upon

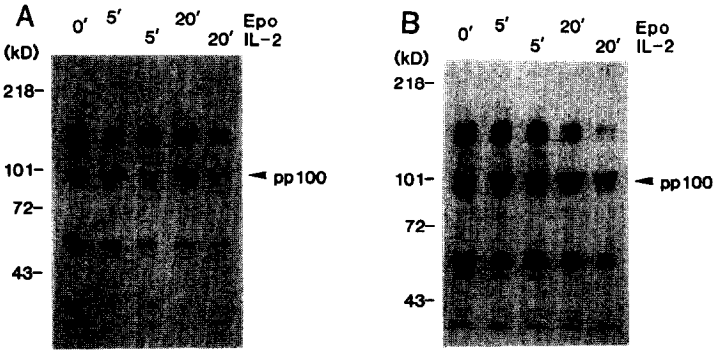

Figure 5. Time course study of Epo- and IL-2-induced protein tyrosine phosphorylation in ELM-I-1 (A) and ELM-I-1β1-18 (B) cells. Cells were exposed to 2 U/ml of Epo or 8 nM of IL-2 for the indicated time and then were lysed and immunoblotted as described in Materials and Methods.

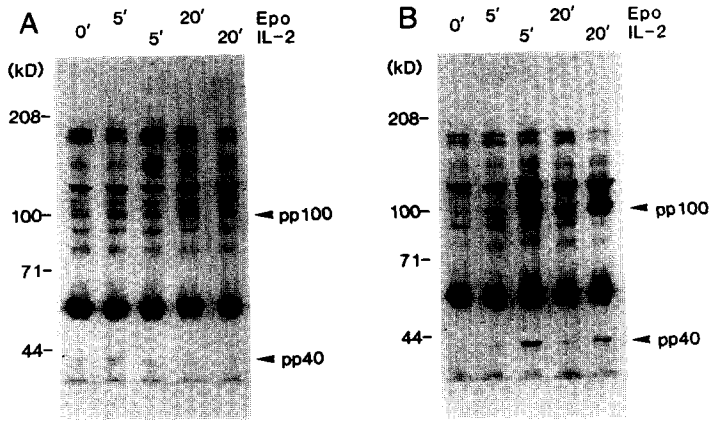

Figure 6. Time course study of Epo- and IL-2-induced protein tyrosine phosphorylation in EP-FDC-P2 (A) and EP-FDC-P2β3-2 (B) cells. Both cells were pre-starved of Epo for 2 hours. Cells were exposed to 2 U/ml of Epo or 8 nM of IL-2 for the indicated time and then were lysed and immunoblotted as described in Materials and Methods. Epo and IL-2 induced tyrosine phosphorylation of proteins with MW of 100 and 40 kD (designated pp100 and pp40,respectively) in EP-FDC-P2β3-2 cells, whereas in EP-FDC-P2 cells only Epo induced tyrosine phosphorylation.

EpoR cDNA transfection (15) and to an IL-2-dependent state upon IL-2R β chain cDNA transfection (16). We also demonstrated that Epo and IL-2 induced the same proliferative response (data not shown) and the same pattern of tyrosine phosphorylation (Figure 6) in EP-FDC-P2 cells (which proliferate in response to Epo or IL-3) (17) transfected with a human IL-2R β chain cDNA (EP-FDC-P2β3-2). Taken these data with our results concerning erythroid differentiation, it is more likely that the Epo and IL-2 receptor systems share a common signal transduction pathway.

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