

Cell type-specific tyrosine phosphorylation of IL-2 receptor β chain in response to IL-2

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Functional activities of the IL-2 receptor (IL-2R) β chain exogenously expressed on lymphoid and non-lymphoid cells were examined in terms of phosphorylation of IL-2R β and cell growth. Lymphoid MOLT-4 and its transfectants expressing IL-2R β either alone or with IL-2R α chain were found to be rapidly phosphorylated predominantly at tyrosine residues of IL-2R β and to be affected in their growth in an IL-2-dependent manner. In contrast, IL-2 induced neither phosphorylation of IL-2R β nor cell growth in non-lymphoid transfectants derived from COS7, HeLa and L929, even though they acquired the IL-2 binding ability when coexpressed as IL-2R β and IL-2R α . These results suggest that IL-2 induces activation of a tyrosine kinase possibly associated with IL-2R β in a cell type-specific manner.

Interleukin-2 receptor β chain; Tyrosine kinase

1. INTRODUCTION

IL-2 was first defined as a T cell growth factor and it is physiologically involved in clonal proliferation of T lymphocytes in an antigen-specific manner. IL-2-mediated growth signals are transduced intracellularly via IL-2R, which contains at least two distinct subunits, α and β chains [1]. Recent studies demonstrated that IL-2R β expressed on lymphoid and oligodendrogloma cell lines binds IL-2 and transduces growth signals [2–5]. In contrast, IL-2R β solely expressed on fibroblastoid cell lines shows no IL-2 binding ability [6,7]. Furthermore, IL-2-mediated growth signals were not demonstrated in fibroblastoid cell lines even though IL-2R β transfected with IL-2R α generates the high-affinity receptor [7,8]. These observations suggest that a cell type-specific factor is required for the formation of a functional receptor.

Accumulating evidence indicates that IL-2 induces activation of a tyrosine kinase which phosphorylates IL-2R β , and suggests that the activation associates with IL-2-mediated growth signal transduction [9–11]. Recently, p56^{lck} and p53/56^{lyn}, cytoplasmic tyrosine kinases, have been shown to be physically associated with

IL-2R β and to be activated by IL-2 stimulation [12–14], although it remains to be clarified which tyrosine kinase plays a crucial role in the IL-2-mediated signal transduction. In the present study, we examined IL-2-induced cell growth and tyrosine phosphorylation of IL-2R β in various cell lines including non-lymphoid cell lines transfected with IL-2R β , and found that cell growth and the tyrosine phosphorylation of IL-2R β are induced in the lymphoid transfectants but not in the fibroblastoid and epitheloid transfectants.

2. MATERIALS AND METHODS

2.1. Cell lines and monoclonal antibody

The cell lines used were a human T-cell leukemia line, MOLT-4, a HTLV-I-carrying human T cell line, MT-1, a simian fibroblastoid cell line, COS7, a human epitheloid cell line, HeLa, and a murine fibroblast cell line, L929. The other cell line, LCT-4 is a subline of L929, which was established by transfection with human cDNA of IL-2R α [15]. TU11 mAb (IgG1), specific for human IL-2R β , does not interfere with IL-2 binding to IL-2R β [16].

2.2. Stable introduction of expression vectors into cell lines

Expression vectors for human IL-2R α (pSRA4) and β (pSRB5) chains were described elsewhere [17]. MOLT-4, MT-1, COS7, HeLa, L929 and LCT-4 cells were transfected with pSRB5, and HeLa cells were co-transfected with pSRB5 and pSRA4. pSRB5 was introduced into MOLT-4 and COS7 subclones which had been stably transfected with pBEH IL-2R α [18], establishing subclones expressing both IL-2R α and β .

2.3. IL-2 binding assay

Human recombinant IL-2 (obtained from Shionogi) was labeled with [¹²⁵I]Na (Amersham) by the chloramine T method, and used for IL-2 binding assay of various cells as described previously [17]. IL-2 binding was analyzed by Scatchard plot.

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Abbreviations: IL-2R, interleukin-2 receptor; FCS, fetal calf serum; BSA, bovine serum albumin; mAb, monoclonal antibody; 2D PAGE, two-dimensional polyacrylamide gel electrophoresis.

2.4. [^3H]/thymidine incorporation assay

A total of 5×10^3 cells of MT-1 or its derivative per well in 96-well plates was cultured in the presence of IL-2 at indicated concentrations in the medium containing 10% FCS and 20 mM HEPES (pH 7.5) for 72 h in triplicate. The medium containing 0.05% BSA and 20 mM HEPES was used for transfectants from MOLT-4, COS7, HeLa and L929. [^3H]TdR (Amersham) was added at 1.0 μCi per well 4 h before termination of incubation. The incorporated [^3H]TdR was counted as described elsewhere [19].

2.5. *In vivo* phosphorylation of IL-2R β

Cells were labeled with [^{32}P]orthophosphate (1.0 mCi/ml, Amersham) for 2 h, and further cultured for indicated times after IL-2 addition. Subsequently, they were lysed and immunoprecipitated with TU11 mAb and Protein A Sepharose CL-4B pretreated with a rabbit anti-mouse IgG antibody (Zymed) as described previously [9]. Samples were then analyzed by 2D PAGE.

2.6. Phosphoamino acid analysis

Gel pieces corresponding to [^{32}P]-labeled IL-2R β spots were excised from the slab gels, and subjected to phosphoamino acid analysis as previously described [9]. Phosphoamino acid markers were detected by ninhydrin staining. Phosphorylated tyrosine, serine and threonine were quantitated with a Bio-image analyzer BAS 2000 (Fuji Film) [20].

3. RESULTS AND DISCUSSION

We established transfectant cell lines expressing IL-2R β which initially did not express endogenous IL-2R β . MOLT $\alpha\beta$ -4, COS $\alpha\beta$ -5 and HeLa $\alpha\beta$ -73 are subclones of MOLT-4, COS7 and HeLa transfectants with both IL-2R α and β cDNAs, respectively. MOLT β -11, MT β -1, COS β -2, HeLa β -1, La β -2 and L β -1 are subclones of MOLT-4, MT-1, COS7, HeLa, LCT-4 and L929 transfectants with IL-2R β cDNA alone, respectively. The transfectant clones were examined for their [^{125}I]IL-2 binding abilities. Apparent numbers of IL-2 binding sites and IL-2 binding affinities of IL-2R on the cell surfaces were calculated by Scatchard plot (Table I). Fibroblastoid COS β -2 and L β -1, and epitheloid HeLa β -1 cells are not able to bind IL-2.

We determined whether IL-2-induced tyrosine phosphorylation of IL-2R β is cell type-specific. In

MOLT $\alpha\beta$ -4 and MT β -1, phosphorylation of the IL-2R β molecules increased during the IL-2 stimulation over 30 min (Fig. 1A,B). On the other hand, with the cell lysate of COS $\alpha\beta$ -5, La β -2 and HeLa $\alpha\beta$ -73, phosphorylated IL-2R β spots were detected before IL-2 stimulation, but little, if any, increase in density of the spots was seen after IL-2 stimulation (Fig. 1C and data not shown). The phosphorylated IL-2R β molecules were further examined for their phosphoamino acid contents. Before IL-2 stimulation, phosphoserine and phosphothreonine of IL-2R β appeared as dominant and minor spots, respectively, but phosphotyrosine of IL-2R β was not appreciably detected in MOLT $\alpha\beta$ -4 and MT β -1. Phosphotyrosine became detectable as early as 1 min after IL-2 stimulation, and reached the maximum level within 15 min of IL-2 stimulation, and then gradually decreased in MOLT $\alpha\beta$ -4 and MT β -1 (Fig. 1A,B). No apparent increase in phosphoserine and phosphothreonine was seen at 1 min after IL-2 stimulation when the tyrosine residues were phosphorylated considerably (Fig. 1A,B). A similar result of phosphoamino acid analysis of IL-2R β was seen with MOLT β -11 [20]. These results are consistent with those obtained with normal activated T cells and IL-2-dependent cell line cells [9–11]. In contrast, no significant increase in the level of phosphotyrosine, phosphothreonine and phosphoserine of IL-2R β was observed during the IL-2 stimulation for at least 30 min in COS $\alpha\beta$ -5 (Fig. 1C), and in HeLa $\alpha\beta$ -73 and La β -2 (data not shown). On the other hand, the amount of phosphotyrosine of IL-2R β profoundly increased in proportion to concentrations of IL-2 in MOLT $\alpha\beta$ -4, MOLT β -11 and MT β -1 but not in COS $\alpha\beta$ -5. In the IL-2-responsive cells, tyrosine phosphorylation of IL-2R β was mediated by the intermediate- and high-affinity receptors but not the low-affinity receptor (data not shown). These results show that the IL-2-induced tyrosine phosphorylation of IL-2R β is mediated by a signal from IL-2R β , and is dependent on cell types.

Table I
Properties of IL-2 binding sites of the transfectants

Transfectants	IL-2 binding					
	High-affinity		Intermediate-affinity		Low-affinity	
	Sites/cell	K_d (pM)	Sites/cell	K_d (nM)	Sites/cell	K_d (nM)
MOLT $\alpha\beta$ -4	1,230	120	3,085	2.3	ND	ND
MOLT β -11	ND*	ND	3,720	1.7	ND	ND
MT β -1	7,100	190	ND	ND	72,000	30
COS $\alpha\beta$ -5	6,736	557	ND	ND	ND	ND
HeLa $\alpha\beta$ -73	252	140	ND	ND	5,312	18.6
La β -2	4,840	608	ND	ND	ND	ND
COS β -2	ND	ND	ND	ND	ND	ND
HeLa β -1	ND	ND	ND	ND	ND	ND
L β -1	ND	ND	ND	ND	ND	ND

* ND, not detected

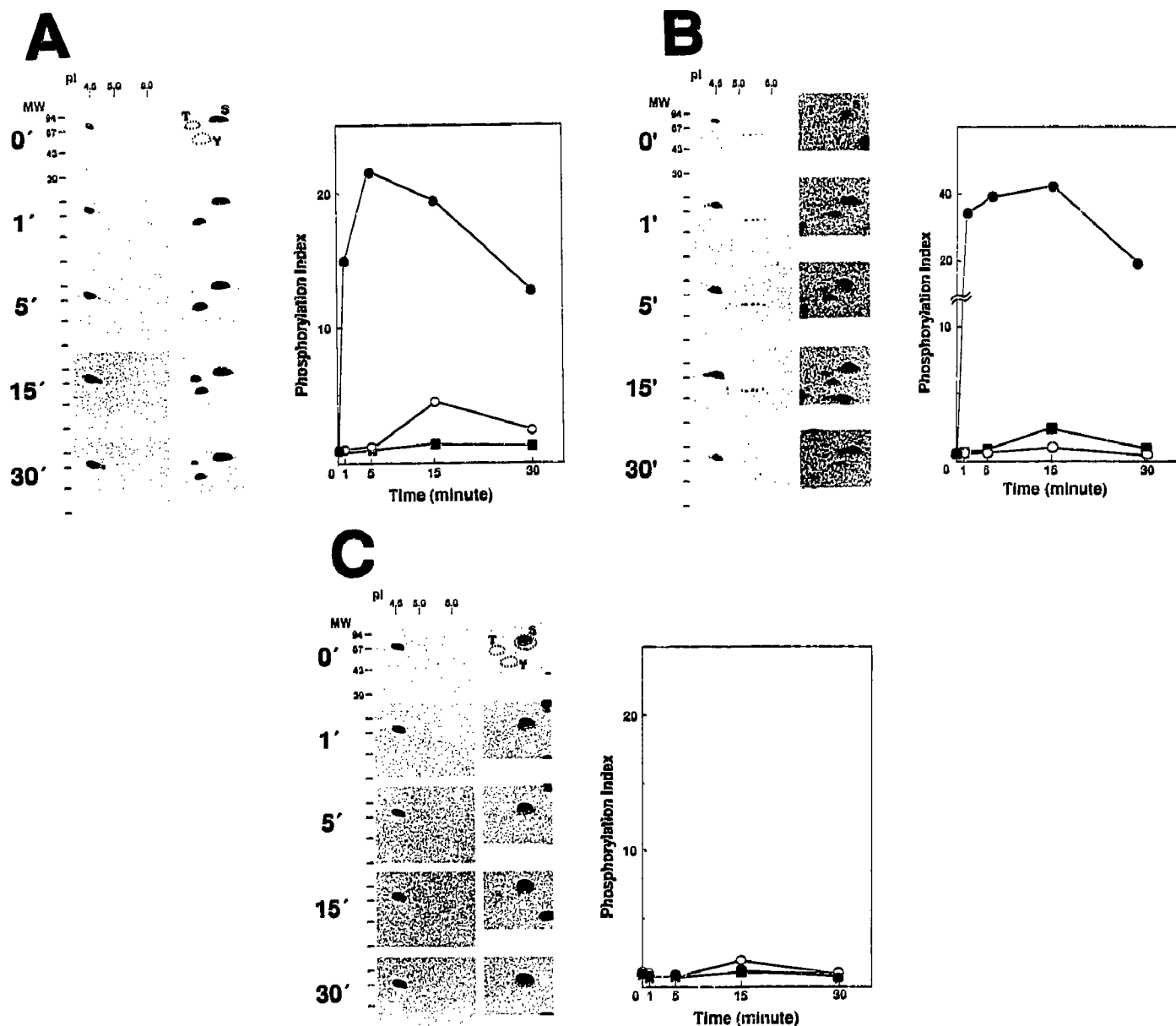


Fig. 1. Phosphorylation of IL-2R β in response to IL-2. The cell lysates of MOLT $\alpha\beta$ -4 (A), MT β -1 (B) and COS $\alpha\beta$ -5 (C) were prepared at indicated times after treatment with 30 nM IL-2. The lysates were immunoprecipitated with TU11 mAb, and subjected to 2D PAGE (left panels). The spots corresponding to each phosphorylated IL-2R β were excised, and analyzed for phosphoamino acids (center panels). Positions of phosphoserine (S), phosphothreonine (T) and phosphotyrosine (Y) are indicated. Each spot for phosphorylated tyrosine, serine and threonine of IL-2R β was quantitated for their radioactivity with BAS2000, and the relative amounts of the phosphotyrosine (●), phosphothreonine (○), and phosphoserine (■) are presented as phosphorylation indexes of ratios of the amount at indicated times to that at 0 min (right panels).

We further examined whether IL-2R expressed on the transfectants could function as a cell growth signal transducer in response to IL-2. In MOLT $\alpha\beta$ -4 and MOLT β -11, IL-2 promoted cell growth in an IL-2 dose-dependent manner under the serum-depleted culture condition (Fig. 2A). The cell growth of MT β -1, but not parental MT-1, was significantly inhibited by the addition of IL-2 as previously reported [5] (Fig. 2B). In contrast, although COS $\alpha\beta$ -5 expressed the high-affinity IL-2R, no effect of IL-2 was observed (Fig. 2C). The

other non-lymphoid transfectants such as HeLa $\alpha\beta$ -73 and L $\alpha\beta$ -2, which expressed the high-affinity IL-2 receptor, did not respond to IL-2 (data not shown). Taken all together, exogenously expressed IL-2R β functions for signal transduction in a cell type-specific manner, correlated with the tyrosine phosphorylation of IL-2R β . These results support the notion that tyrosine phosphorylation of IL-2R β is one of the earliest biochemical reactions in the IL-2-induced cell growth signaling.

We [20] and others [21] previously demonstrated that

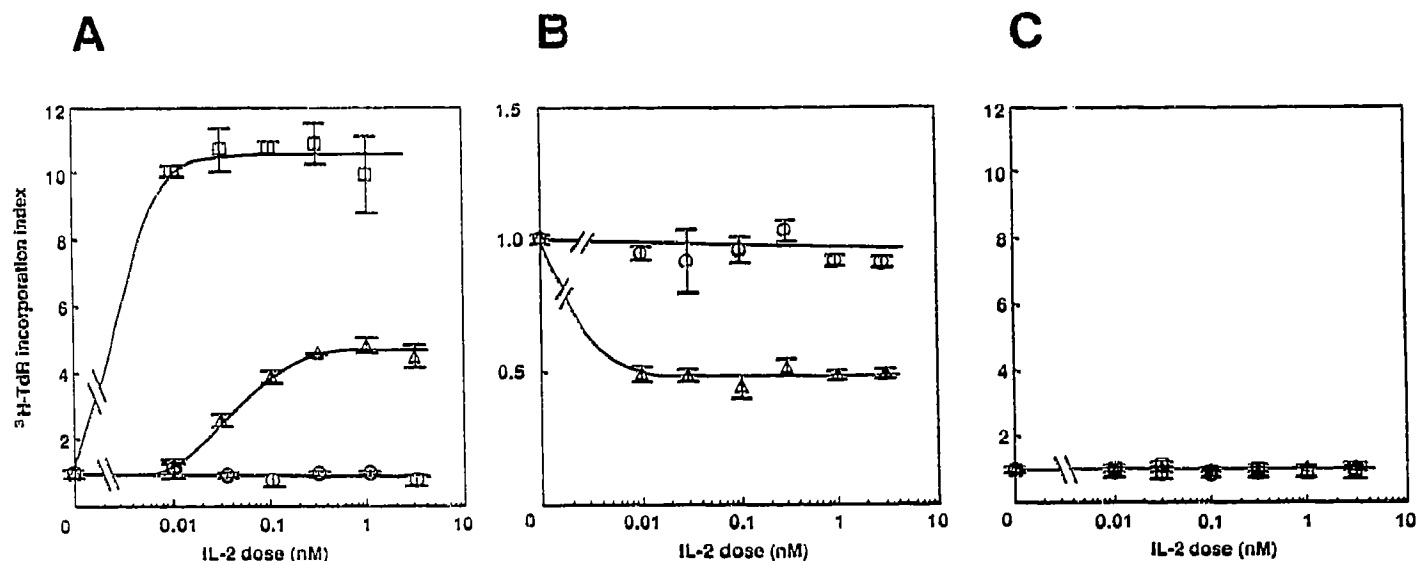


Fig. 2. Proliferative responses of the transfectants to IL-2. Cells were cultured with various concentrations of IL-2 and measured for incorporation of [3 H]TdR in triplicate. [3 H]TdR incorporation indexes represent the ratios of radioactivity of incorporated [3 H]TdR in the presence of indicated IL-2 doses to that in the absence of IL-2. (A) 5×10^3 cells of MOLT-4 ($\circ$), MOLT β -11 (Δ) and MOLT $\alpha\beta$ -4 ($\square$) in the medium containing 0.05% BSA without IL-2, gave [3 H]TdR incorporation of $1,488 \pm 101$, $2,883 \pm 555$ and $2,825 \pm 419$ cpm $\pm$ S.E.M., respectively. (B) 5×10^3 cells of MT-1 ($\circ$) and MT β -1 (Δ) incorporated [3 H]TdR of $48,511 \pm 985$ and $43,165 \pm 1,865$ cpm $\pm$ S.E.M., respectively, in the medium containing 10% FCS without IL-2. (C) The 0.05% BSA medium was also used for 5×10^3 cell cultures of COS7 ($\circ$), COS β -2 (Δ) and COS $\alpha\beta$ -5 ($\square$) which incorporated [3 H]TdR of $8,983 \pm 868$, $4,076 \pm 81$ and $3,818 \pm 625$ cpm $\pm$ S.E.M., respectively, without IL-2. Each plot represents the mean value $\pm$ S.E.M. of incorporated [3 H]TdR in triplicate. The same experiments were repeated more than three times, and very similar results were obtained. Figures represent typical results.

a tyrosine kinase activity, which mediated phosphorylation of IL-2R β in vitro, was co-precipitated with IL-2R β . Collectively, a putative tyrosine kinase associated with IL-2R β might be a molecule responsible for determining the cell-type specificity in the IL-2-mediated signal transduction. So far, p56^{lck} and p53/56^{lyn}, nonreceptor type tyrosine kinases, have been shown to be possible candidates for the tyrosine kinases involved in IL-2-induced signal transduction in T cell lines and a B cell line, respectively [12–14]. However, p56^{lck} negative T cell lines such as MT-2, HUT102 and TCL-Kan expressing IL-2R α and β endogenously, were also found to phosphorylate tyrosine residues of IL-2R β in response to IL-2 ([20] and our unpublished observations). Thereby, a tyrosine kinase other than p56^{lck} may also be associated with IL-2R β and phosphorylate tyrosine residues of IL-2R β upon IL-2 stimulation. On the other hand, we recently identified the γ chain of IL-2R, of which expression was detected specifically on lymphoid cells, but not on non-lymphoid cells such as HeLa, COS7 and L929 cells [22]. Our preliminary experiments showed that IL-2-induced tyrosine phosphorylation of IL-2R β was detected in L $\alpha\beta$ -2-derived clone transfected with the γ chain gene, suggesting the possibility that IL-2R γ but not tyrosine kinase is responsible for determining the cell-type specificity in the IL-2-mediated signal transduction (Asao et al., unpublished observations). All together, it may be that a tyrosine kinase associated with IL-2R β is exchangeable among some

members of the tyrosine kinase family. Alternatively, a common tyrosine kinase associated with IL-2R may exist in various types of cells including fibroblastoid cells. Further studies of IL-2R γ and tyrosine kinase(s) associated with IL-2R β will give us a better understanding about the IL-2-induced signal transduction.

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