Volume 310, number 1, 22–26 FEBS 11556 September 1992

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

Satoru Kumaki[a], Hironobu Asao[a], Toshikazu Takeshita[a], Yumiyo Kurahayashi[a], Masataka Nakamura[a], Thomas Beckers[b], Joachim W. Engels[b] and Kazuo Sugamura[a]

[a]*Department of Microbiology, Tohoku University, School of Medicine, 2-1 Seiryo-machi, Aoba-Ku, Sendai 980, Japan* and [b]*Institut für Organische Chemie, Universität Frankfurt, Niederurseler Hang, 6000 Frankfurt/Main 50, Germany*

Received 30 July 1992

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-2Rβ and cell growth. Lymphoid MOLT-4 and its transfectants expressing IL-2Rβ either alone or with IL-2R α chain were found to be rapidly phosphorylated predominantly at tyrosine residues of IL-2Rβ and to be affected in their growth in an IL-2-dependent manner. In contrast, IL-2 induced neither phosphorylation of IL-2Rβ 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-2Rβ and IL-2Rα. These results suggest that IL-2 induces activation of a tyrosine kinase possibly associated with IL-2Rβ 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-2Rβ expressed on lymphoid and oligodendroglioma cell lines binds IL-2 and transduces growth signals [2–5]. In contrast, IL-2Rβ 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-2Rβ transfected with IL-2Rα 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-2Rβ, 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-2Rβ 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-2Rβ in various cell lines including non-lymphoid cell lines transfected with IL-2Rβ, and found that cell growth and the tyrosine phosphorylation of IL-2Rβ are induced in the lymphoid transfectants but not in the fibroblastoid and epitheloid transfectants.

Correspondence address: K. Sugamura, Department of Microbiology, Tohoku University, School of Medicine, 2-1 Seiryo-machi, Aoba-Ku, Sendai 980, Japan.

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. 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-2Rα [15]. TU11 mAb (IgG1), specific for human IL-2Rβ, does not interfere with IL-2 binding to IL-2Rβ [16].

2.2. *Stable introduction of expression vectors into cell lines*

Expression vectors for human IL-2Rα (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-2Rα [18], establishing subclones expressing both IL-2Rα and β.

2.3. *IL-2 binding assay*

Human recombinant IL-2 (obtained from Shionogi) was labeled with [^{125}I]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.

Published by Elsevier Science Publishers B.V.

2.4. *[^{3}H]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. [^{3}H]TdR (Amersham) was added at 1.0 μCi per well 4 h before termination of incubation. The incorporated [^{3}H]TdR was counted as described elsewhere [19].

2.5. *In vivo phosphorylation of IL-2Rβ*

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-2Rβ 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-2Rβ which initially did not express endogenous IL-2Rβ. MOLTαβ-4, COSαβ-5 and HeLaαβ-73 are subclones of MOLT-4, COS7 and HeLa transfectants with both IL-2Rα and β cDNAs, respectively. MOLTβ-11, MTβ-1, COSβ-2, HeLaβ-1, Lαβ-2 and Lβ-1 are subclones of MOLT-4, MT-1, COS7, HeLa, LCT-4 and L929 transfectants with IL-2Rβ 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 COSβ-2 and Lβ-1, and epitheloid HeLaβ-1 cells are not able to bind IL-2.

We determined whether IL-2-induced tyrosine phosphorylation of IL-2Rβ is cell type-specific. In MOLTαβ-4 and MTβ-1, phosphorylation of the IL-2Rβ molecules increased during the IL-2 stimulation over 30 min (Fig. 1A,B). On the other hand, with the cell lysate of COSαβ-5, Lαβ-2 and HeLaαβ-73, phosphorylated IL-2Rβ 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-2Rβ molecules were further examined for their phosphoamino acid contents. Before IL-2 stimulation, phosphoserine and phosphothreonine of IL-2Rβ appeared as dominant and minor spots, respectively, but phosphotyrosine of IL-2Rβ was not appreciably detected in MOLTαβ-4 and MTβ-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 MOLTαβ-4 and MTβ-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-2Rβ was seen with MOLTβ-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-2Rβ was observed during the IL-2 stimulation for at least 30 min in COSαβ-5 (Fig. 1C), and in HeLaαβ-73 and Lαβ-2 (data not shown). On the other hand, the amount of phosphotyrosine of IL-2Rβ profoundly increased in proportion to concentrations of IL-2 in MOLTαβ-4, MOLTβ-11 and MTβ-1 but not in COSαβ-5. In the IL-2-responsive cells, tyrosine phosphorylation of IL-2Rβ 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-2Rβ is mediated by a signal from IL-2Rβ, 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)
MOLTαβ-4	1,230	120	3,085	2.3	ND	ND
MOLTβ-11	ND*	ND	3,720	1.7	ND	ND
MTβ-1	7,100	190	ND	ND	72,000	30
COSαβ-5	6,736	557	ND	ND	ND	ND
HeLaαβ-73	252	140	ND	ND	5,312	18.6
Lαβ-2	4,840	608	ND	ND	ND	ND
COSβ-2	ND	ND	ND	ND	ND	ND
HeLaβ-1	ND	ND	ND	ND	ND	ND
Lβ-1	ND	ND	ND	ND	ND	ND

* ND, not detected

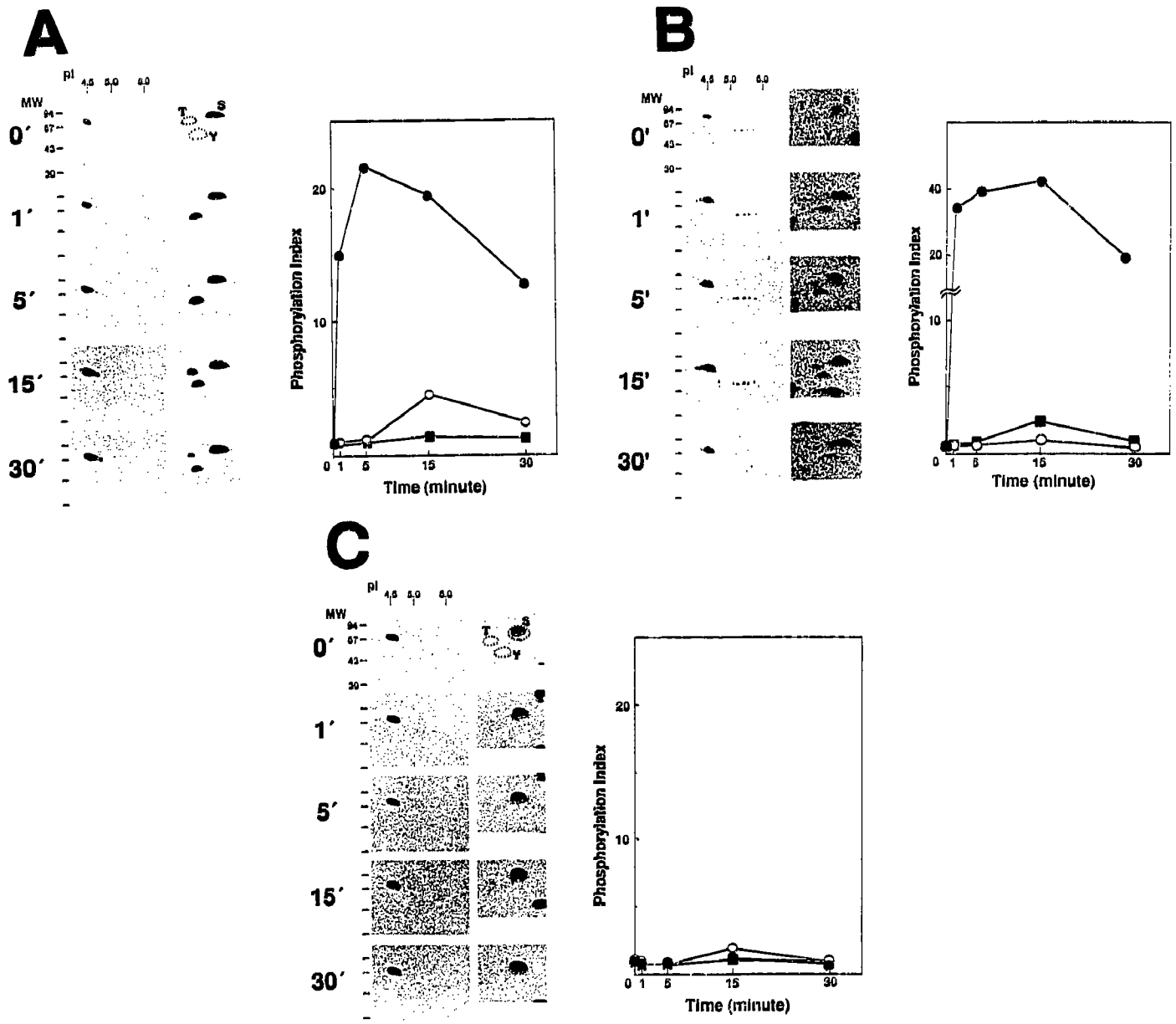

Fig. 1. Phosphorylation of IL-2Rβ in response to IL-2. The cell lysates of MOLTαβ-4 (A), MTβ-1 (B) and COSαβ-5 (C) were prepared at indicated times after treatment with 30 nM IL-2. The lysates were immunoprecipitated with TU11 mAb, and subjected to 2D PAGE (left panels). The spots corresponding to each phosphorylated IL-2Rβ 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-2Rβ 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 MOLTαβ-4 and MOLTβ-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 MTβ-1, but not parental MT-1, was significantly inhibited by the addition of IL-2 as previously reported [5] (Fig. 2B). In contrast, although COSαβ-5 expressed the high-affinity IL-2R, no effect of IL-2 was observed (Fig. 2C). The other non-lymphoid transfectants such as HeLaαβ-73 and Lαβ-2, which expressed the high-affinity IL-2 receptor, did not respond to IL-2 (data not shown). Taken all together, exogenously expressed IL-2Rβ functions for signal transduction in a cell type-specific manner, correlated with the tyrosine phosphorylation of IL-2Rβ. These results support the notion that tyrosine phosphorylation of IL-2Rβ is one of the earliest biochemical reactions in the IL-2-induced cell growth signaling.

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

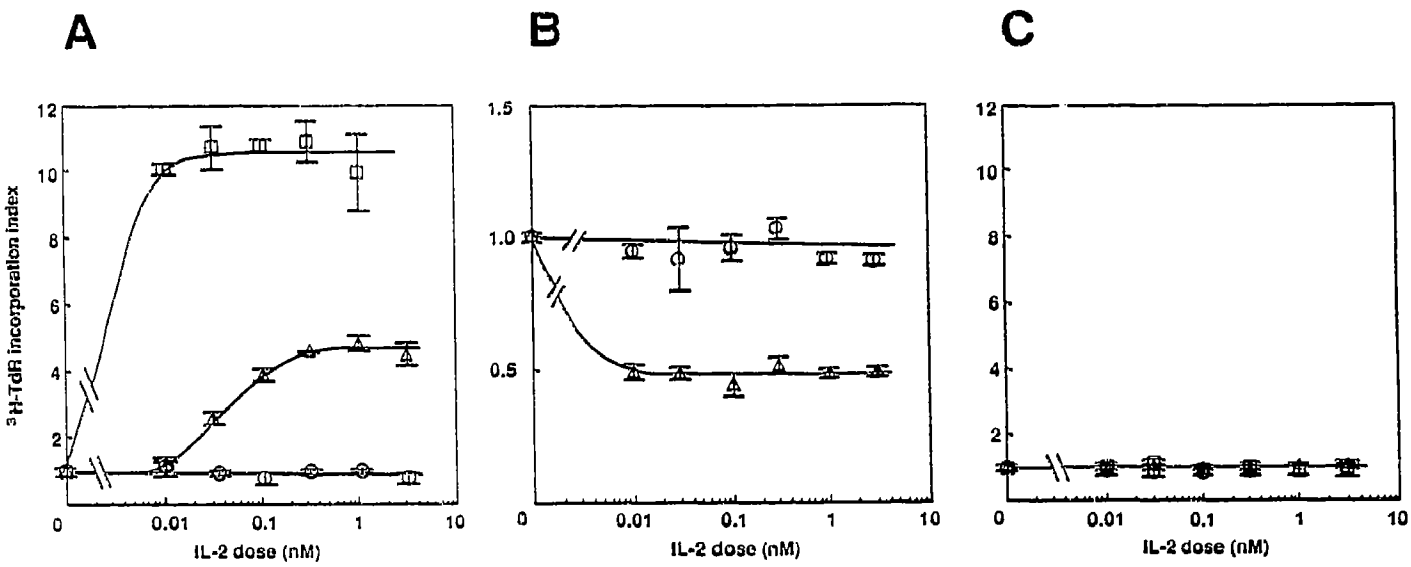

Fig. 2. Proliferative responses of the transfectants to IL-2. Cells were cultured with various concentrations of IL-2 and measured for incorporation of [^{3}H]TdR in triplicate. [^{3}H]TdR incorporation indexes represent the ratios of radioactivity of incorporated [^{3}H]TdR in the presence of indicated IL-2 doses to that in the absence of IL-2. (A) 5×10^3 cells of MOLT-4 (○), MOLTβ-11 (△) and MOLT$\alpha\beta$-4 (□) in the medium containing 0.05% BSA without IL-2, gave [^{3}H]TdR incorporation of 1,488 ± 101, 2,883 ± 555 and 2,825 ± 419 cpm ± S.E.M., respectively. (B) 5×10^3 cells of MT-1 (○) and MTβ-1 (△) incorporated [^{3}H]TdR of 48,511 ± 985 and 43,165 ± 1,865 cpm ± 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 (○), COSβ-2 (△) and COS$\alpha\beta$-5 (□) which incorporated [^{3}H]TdR of 8,983 ±868, 4,076 ± 81 and 3,818 ± 625 cpm ± S.E.M., respectively, without IL-2. Each plot represents the mean value ± 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-2Rβ in vitro. was co-precipitated with IL-2Rβ. Collectively, a putative tyrosine kinase associated with IL-2Rβ 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-2Rα and β endogenously, were also found to phosphorylate tyrosine residues of IL-2Rβ in response to IL-2 ([20] and our unpublished observations). Thereby, a tyrosine kinase other than p56^{lck} may also be associated with IL-2Rβ and phosphorylate tyrosine residues of IL-2Rβ 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-2Rβ was detected in L$\alpha\beta$-2-derived clone transfected with the γ chain gene, suggesting the possibility that IL-2Rγ 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-2Rβ 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-2Rγ and tyrosine kinase(s) associated with IL-2Rβ will give us a better understanding about the IL-2-induced signal transduction.

Acknowledgements: We thank Drs. T. Taniguchi and M. Daley for providing the cell line, LCT-4 and for critical reading of this manuscript, respectively. This work was supported in part by Grants-in-Aid for General Scientific Research and Cancer Research from the Ministry of Education, Science and Culture, and by grants from Special Coordination Funds of the Science and Technology Agency of the Japanese Government, from the Yamada Science Foundation, from the Takeda Scientific foundation and from the Naito Foundation.

REFERENCES

[1] Smith, K.A. (1988) Science 240, 1169–1176.
[2] Tanaka, T., Tsudo, M., Karasuyama, H., Toyama, N., Hatakeyama, M., Taniguchi, T. and Miyasaka, M. (1991) Int. Immunol. 3, 105–108.
[3] Okamoto, Y., Minamoto, S., Shimizu, K., Mogami, H. and Taniguchi, T. (1990) Proc. Natl. Acad. Sci. USA 87, 6584–6588.
[4] Hatakeyama, M., Mori, H., Doi, T. and Taniguchi, T. (1989) Cell 59, 837–845.
[5] Tsudo, M., Karasuyama, H., Kitamura, F., Nagasaka, Y., Tanaka, T. and Miyasaka, M. (1989) J. Immunol. 143, 4039–4043.
[6] Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. and Taniguchi, T. (1989) Science 244, 551–556.

[7] Minamoto, S., Mori, H., Hatakeyama, M., Kono, T., Doi, T., Ide, T., Uede, T. and Taniguchi, T. (1990) J. Immunol. 145, 2177–2182.
[8] Tsudo, M., Karasuyama, H., Kitamura, F., Tanaka, T., Kubo, S., Yamamura, Y., Tamatani, T., Hatakeyama, M., Taniguchi, T. and Miyasaka, M. (1990) J. Immunol. 145, 599–606.
[9] Asao, H., Takeshita, T., Nakamura, M., Nagata, K. and Sugamura, K. (1990) J. Exp. Med. 171, 637–644.
[10] Mills, G.B., May, C., McGill, M., Fung, M., Baker, M., Sutherland, R. and Greene, W.C. (1990) Interleukin 2-induced tyrosine phosphorylation, J. Biol. Chem. 265, 3561–3567.
[11] Merida, I. and Gaulton, G.N. (1990) J. Biol. Chem. 265, 5690–5694.
[12] Horak, I.D., Gress, R.E., Lucas, P.J., Horak, E.M., Waldmann, T.A. and Bolen, J.B. (1991) Proc. Natl. Acad. Sci. USA 88, 1996–2000.
[13] Hatakeyama, M., Kono, T., Kobayashi, N., Kawahara, A., Levin, S.D., Perlmutter, R.M. and Taniguchi, T. (1991) Science 252, 1523–1528.
[14] Torigoe, T., Saragovi, H.U. and Reed, J.C. (1992) Proc. Natl. Acad. Sci. USA 89, 2674–2678.
[15] Hatakeyama, M., Minamoto, S., Uchiyama, T., Hardy, R.R., Yamada, G. and Taniguchi, T. (1985) Nature 318, 467–470.
[16] Suzuki, J., Takeshita, T., Ohbo, K., Asao, H., Tada, K. and Sugamura, K. (1989) Int. Immunol. 1, 373–377.
[17] Takeshita, T., Ohtani, K., Asao, H., Kumaki, S., Nakamura, M. and Sugamura, K. (1992) J. Immunol. 148, 2154–2158.
[18] Beckers, T., Hauser, H.J., Hüsken, D. and Engels, J.W. (1988) J. Biol. Chem. 263, 8359–8365.
[19] Takeshita, T., Goto, Y., Tada, K., Nagata, K., Asao, H. and Sugamura, K. (1989) J. Exp. Med. 169, 1323–1332.
[20] Asao, H., Kumaki, S., Takeshita, T., Nakamura, M. and Sugamura, K. (1992) FEBS Lett. 304, 141–145.
[21] Fung, M.R., Scearce, R.M., Hoffman, J.A., Peffer, N.J., Hammes, S.R., Hosking, J.B., Shumandt, R., Kuziel, W.A., Haynes, B.F., Mills, G.B. and Greene, W.C. (1991) J. Immunol. 147, 1253–1260.
[22] Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Nakamura, M. and Sugamura, K. (1992) Science 257, 379–382.