The IL-2/IL-2 Receptor System : Involvement of a Novel Receptor Subunit, γ Chain, in Growth Signal Transduction

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SUGAMURA, K., TAKESHITA, T., ASAO, H., KUMAKI, S., OHBO, K., OHTANI, K. and NAKAMURA, M. *The IL-2/IL-2 Receptor System : Involvement of a Novel Receptor Subunit, γ Chain, in Growth Signal Transduction.* Tohoku J. Exp. Med., 1992, 168 (2), 231-237 — We previously demonstrated the existence of a third component, p64, of IL-2 receptor (IL-2R), tentatively named the γ chain of IL-2R. Our recent studies provided evidence suggesting that the γ chain endows the β chain of IL-2R with IL-2 binding ability. The γ chain was detected in lymphoid transfectants of IL-2R β cDNA, which showed the intermediate-affinity IL-2R, but not in nonlymphoid transfectants of IL-2R β cDNA, which showed no IL-2 binding activity. The comparative study between two subclones of lymphoid MOLT4 transfectant of IL-2R β cDNA demonstrated that the amount of the γ chain coprecipitated with IL-2R β was proportional to numbers of the IL-2 binding sites. These results suggest the possibility that the γ chain associates with IL-2R β and has an important role in formation of the intermediate-affinity IL-2R complex. On the other hand, we have also demonstrated the association of IL-2R β with a certain tyrosine kinase, of which activation by IL-2 could be indispensable process at the initial pathway of signal transduction. ——— γ -chain ; IL-2 ; IL-2 receptor ; signal transduction ; tyrosine kinase

The IL-2 receptor (IL-2R) is classified into the three types differing in IL-2 binding affinities: high-affinity (dissociation constant, $K_d=10^{-11}$ M), intermediate-affinity ($K_d=10^{-9}$ M) and low-affinity ($K_d=10^{-8}$ M), consisting of a complex of α and β chains, the β chain or the α chain, respectively (Smith 1988). The transfection experiments with α and β genes demonstrated that lymphoid transfectants of β gene express the intermediate-affinity receptor and transduce IL-2-mediated signals, and transfectants of α gene express the low-affinity receptor but cannot transduce IL-2-mediated signals (Leonard et al. 1984 ; Nikaido et al. 1984 ; Hatakeyama et al. 1989). However, the β chain is not functional for IL-2 binding when it is expressed on fibroblast cells (Hatakeyama et al. 1989). These findings suggest that there are cell type-specific components associated with

the β chain that result in IL-2 binding. We previously demonstrated the existence of a novel component, p64, tentatively named the γ chain of IL-2R, which was coprecipitated with the β chain (Takeshita et al. 1990), and recently obtained the evidence for biological significance of the γ chain (Takeshita et al. 1992).

Biological significance of IL-2R γ

We examined the biological significance of the γ chain by using various transfectants of α and/or β genes. They are derived from lymphoid MOLT-4 and Raji cell lines, and epitheloid HeLa cell line and fibroblast COS-7 cell line. The affinities and sites of IL-2 receptor expressed on these transfectants were calculated from Scatchard plots of IL-2 bindings. Transfectants, MOLT $\alpha\beta$, Raji $\alpha\beta$, HeLa $\alpha\beta$ and COS $\alpha\beta$, of both α and β genes, express the high-affinity receptor irrespective of lymphoid and nonlymphoid cells. However, of the β gene transfectants, lymphoid MOLT β and Raji β express the intermediate-affinity receptor, but nonlymphoid HeLa β and COS β transfectants have no IL-2 binding ability. We then examined these transfectants, especially MOLT β , Raji β , HeLa $\alpha\beta$ and COS $\alpha\beta$ for detection of the γ chain. MOLT β and Raji β cell lysates contained the γ chain but lysates of HeLa $\alpha\beta$ and COS $\alpha\beta$ did not (Fig. 1). MOLT β cells were recloned, and two subclones, MOLT β -11 and MOLT β -12, were obtained. These two transfectant clones were examined for the ability to bind ^{125}I -TU27 mAb specific for IL-2R β (Takeshita et al. 1989) and ^{125}I -IL-2 by Scatchard plots (Fig. 2). The ratio of IL-2 binding sites on MOLT β -11 compared to MOLT β -12 was 0.61, and the number of IL-2R β molecules did not correlate with the number of IL-2 binding sites, suggesting the requirement for another receptor component for IL-2 binding. MOLT β -11 and MOLT β -12 cells were then examined for the

Fig. 1. Detection of IL-2R γ on various transfectant cells with IL-2R α and/or β . Transfectant cells MOLT β , Raji β , HeLa $\alpha\beta$ and COS $\alpha\beta$, were surface-labeled with ^{125}I , and untreated or treated with 30 nM IL-2. Their lysates were immunoprecipitated with anti-IL-2R mAb, TU11. The immunoprecipitates were analyzed on 2-D PAGE.

Fig. 2. Scatchard plot analyses for bindings of ^{125}I -TU27 mAb and ^{125}I -IL-2. Binding assays of ^{125}I -TU27 mAb (upper panel) and ^{125}I -IL-2 (under panel) were carried out for two subclones of MOLT β , MOLT β -11 ($\circ$) and MOLT β -12 ($\bullet$), and their bindings were analyzed by Scatchard plots.

association of the γ chain with IL-2R β . The density of IL-2R γ , coprecipitated with IL-2R β , increased in proportion to IL-2 concentration, and reached a plateau level at 10 nM IL-2 in both MOLT β -11 and MOLT β -12 clones, whereas the density of IL-2R β precipitated was little changed by the IL-2 treatment (Fig. 3). The 1.7 nM IL-2, which gave a half maximal density of IL-2R γ in both the cell clones, gave the same K_d value for IL-2R as calculated in Fig. 3. The ratio of the maximum IL-2R γ densities of MOLT β -11/MOLT β -12 was 0.62, which is similar to the 0.61 the ratio of IL-2 binding sites (MOLT β -11/MOLT β -12). Thus, the

Fig. 3. Detection of IL-2R γ on MOLT β -11 and MOLT β -12 cells. MOLT β -11 and MOLT β -12 cells were surface-labeled with ^{125}I , and untreated or treated with the indicated doses of IL-2. Their lysates were immunoprecipitated with TU11 mAb, and the immunoprecipitates were analyzed by 2D-PAGE (left hand panel). The densities of p64 spots were quantitated with Bio-Image Analyzer BAS-2000, and 100% indicated the densities of the spots at 30 nM IL-2 (right hand panel).

association of IL-2R γ with IL-2R β correlates well with the IL-2 binding ability of IL-2R β (Takeshita et al. 1992).

Association of a tyrosine kinase with IL-2R β

We previously demonstrated that IL-2 induces tyrosine phosphorylation of the β chain (Asao et al. 1990). IL-2-dependent ILT-Mat cells were incubated with ^{32}P -orthophosphate and then stimulated with IL-2 for the indicated times. Their lysates were immunoprecipitated with TU11 mAb specific for IL-2R β (Suzuki et al. 1989), and analyzed on 2-D PAGE. Phosphorylated β chain molecules were then analyzed for phosphoamino acids. The tyrosine phosphorylation of IL-2R β was increased within one minute after stimulation, and the threonine phosphorylation of IL-2R β was also increased but slower than the tyrosine phosphorylation. The serine phosphorylation was little changed. These results suggested that a certain tyrosine kinase is associated with the β chain, and its activation by IL-2 is involved in an early event of signal transduction. To confirm the association of tyrosine kinase with IL-2 receptor, we performed in vitro kinase assays of the IL-2 receptor complex. MOLT β cells were stimulated with IL-2 and then their lysates were immunoprecipitated with TU11 mAb. Subsequently, ^{32}P - γ ATP was added. They were then incubated and analyzed on 2-D PAGE. The β and γ chains were phosphorylated in vitro (Fig. 4A and B). The phosphorylated β and γ chains were then analyzed for

Fig. 4. In vitro phosphorylation of IL-2R β and IL-2R γ . MOLT β cells were untreated (A) or treated (B) with 30 nM IL-2, and their lysates were immunoprecipitated with TU11 mAb. The immunoprecipitates were incubated with [γ ^{32}P]-ATP, and analyzed by 2-D PAGE. In vitro phosphorylated IL-2R β (a and b) and IL-2R γ (c) molecules were analyzed for their phosphoamino acids.

phosphoamino acids (Fig. 4a, b and c). Only tyrosine residues of the β and γ chains were phosphorylated *in vitro*, which indicates the tyrosine kinase in specifically coprecipitated with the β and γ chain complex.

From these studies, we propose the following possibilities that the γ chain functions by inducing conformational changes of IL-2R β which are prerequisite to IL-2 binding, or alternatively that each single molecule of IL-2R γ and IL-2R β has no IL-2 binding activity but the complex consisting of them binds to IL-2 at an intermediate-affinity. Others have also found molecules associated with IL-2R, which included ICAM-I (p95) (Szollosi et al. 1987), HLA class I (p40-42) (Sharon et al. 1988), p22 and p40 (Saragovi and Malek 1990), p95-110 (Colamonici et al. 1990) and p100 (Sharon et al. 1990), but there is no evidence that these molecules contribute to the formation of functional IL-2R complex. In our experimental system with no chemical crosslinker, only the p64 molecule was detected in the complexes of both functional high-affinity IL-2R and functional intermediate-affinity IL-2R.

Our studies also extended previous observations that a certain tyrosine kinase molecule associates with IL-2R β (Saltzman et al. 1988 ; Ferris et al 1989 ; Sharon et al. 1989 ; Asao et al. 1990). The complex consisting of β and γ chains and IL-2 contained tyrosine kinase activity because the β and γ chains were phosphorylated specifically on tyrosine residues *in vitro*. The tyrosine kinase activation by IL-2 showed the same lymphoid tropism as the γ chain detection did, suggesting that the γ chain may be a tyrosine kinase molecule, or may be implicated in tyrosine kinase activation which is essential for signal transduction from IL-2R β . Recent reports showed that p56^{lck}, a non-receptor type tyrosine kinase encoded by a proto-oncogene, is physically associated with IL-2R β , and IL-2 activates the tyrosine kinase of p56^{lck}, which suggests that p56^{lck} may be involved in IL-2-mediated signal transduction *in vivo* (Hatakeyama et al. 1991 ; Horak et al. 1991). However, we have recently demonstrated that IL-2-induced tyrosine phosphorylation of IL-2R β was detected in at least three human T cell lines negative for p56^{lck} in Western blots and Northern blots (Kumaki et al 1992).

Fig. 5. A structural model of IL-2R complexes. The high-affinity and the intermediate-affinity IL-2Rs are composed of complexes consisting of three subunits, α , β and γ , and two subunits, β and γ , respectively, in lymphoid cells. Both β and γ chains are associated with a tyrosine kinase, PTK.

Therefore, a tyrosine kinase other than p56^{lck} may associate with the IL-2R β and participate in growth signal transduction. Based on our present study, we would propose a structural model of IL-2 receptor in Fig. 5. Further molecular and functional characterization of the γ chain and the tyrosine kinase associated with the IL-2R complex should lead to elucidation of mechanisms of signal transduction mediated by IL-2.

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