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Interleukin 2 receptor β chain expressed in an oligodendroglioma line binds interleukin 2 and delivers growth signal

(neuroglia/cytokine/central nervous system/signal transduction)

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ABSTRACT **Interleukin 2 (IL-2) is a potent growth factor for T lymphocytes, playing a crucial role in the immune response. In view of the considerable evidence that the immunoregulatory cytokines (or lymphokines) also play a role in the growth and differentiation of cells in the central nervous system (CNS), we examined the operation of the IL-2 system in a cell line of CNS origin by expressing a cDNA encoding the β chain of the human IL-2 receptor (IL-2Rβ, a 75-kDa protein). When the cDNA was expressed in a human oligodendroglioma cell line, ONS-21, the IL-2Rβ bound IL-2 with an affinity similar to that in lymphoid cells (K_d, ≈2 nM). Furthermore, cell proliferation ([^{3}H]thymidine incorporation) was stimulated by IL-2. These results demonstrate that the same cytokine receptor is functional in cells of the immune system and CNS and point to a molecular mechanism that is similar for growth-signal transduction between lymphoid and neural cells but that may be different in other cells, such as fibroblasts.**

Cytokines are known to play a key role in various aspects of cellular growth and differentiation. In particular, they have been extensively studied in the context of regulation of the immune response. Many of the immunoregulatory cytokines are produced by antigen-activated T lymphocytes (T cells) as well as by other lymphoid cells, and they in turn exert multiple activities on these cells (reviewed in ref. 1). More recently, a number of reports have indicated that neurons and glial cells in the central nervous system (CNS) also respond to immunoregulatory cytokines such as interleukin 1 (IL-1), IL-2, IL-3, IL-6, and interferon γ (2–5). Furthermore, it has been shown that some of the cytokines are produced locally in the brain (2, 4, 6, 7).

IL-2 has been known as the major growth factor for T cells and plays a crucial role in the clonal proliferation of antigen-stimulated T cells (8–10). In T cells, it delivers a growth signal through a specific receptor complex that binds IL-2 with high affinity (K_d, 10–50 pM). The high-affinity IL-2 receptor (IL-2R) complex consists of at least two distinct receptor components, the α chain (IL-2Rα) and the β chain (IL-2Rβ), which manifests low-affinity (K_d, 10 nM) and intermediate affinity (K_d, 1–2 nM), respectively, in the absence of the other chain (10–12). Recent reports indicate that IL-2Rβ but not IL-2Rα can singly deliver the IL-2 signal in T cells and large granular lymphocytes (13–15). On the other hand, a series of cDNA expression studies have revealed that the IL-2Rβ expressed in fibroblast cell lines (e.g., NIH 3T3, L929, and COS-7 cells) cannot bind IL-2 (12, 36). These observations have thus suggested the requirement of an additional component(s) for the receptor function, which may be specific to a certain cell lineage(s) such as cells of lymphoid lineage.

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The role of IL-2 in the CNS has been documented by a number of studies in which IL-2 has been shown to stimulate proliferation of neonatal rat oligodendrocytes and human glioblastoma cell clones at relatively high concentrations (5–50 nM) (16, 17). It has been reported that IL-2 inhibits proliferation of oligodendrocyte precursors (18). The myelin-producing oligodendrocytes extend processes that wrap concentrically around the axons to form an insulating myelin sheath. The myelin sheath is interrupted at regular intervals by the nodes of Ranvier, where axonal electrical excitation is confined (19). In a well defined demyelination disease condition, multiple sclerosis (MS), the number of oligodendrocytes is reduced within the pathological lesion (plaque) but is increased at the plaque margin (20, 21). Furthermore, cells in that plaque margin were found to exhibit positive staining patterns with antibodies specific for IL-2 and IL-2Rα (22). These observations suggest a potential physiological role for IL-2 in the CNS, particularly in the pathology of MS.

A series of observations have thus raised the issue as to whether the IL-2 signal is transduced in these cells by the same IL-2Rs functioning in the immune system. In this study, we report the establishment of cell lines from human oligodendroglioma cells (ONS-21 cells) that share several properties with normal oligodendrocytes. These ONS-21 cells lack any detectable IL-2Rα but express IL-2Rβ at very low levels. Although IL-2 has no detectable effect on the parental ONS-21 cells before and after cloning, the cloned cells were found to respond to IL-2 for proliferation when IL-2Rβ expression was amplified following transfection with IL-2Rβ cDNA expression vectors. These results will be discussed in light of the mechanism of IL-2 signal transduction via the IL-2Rβ in lymphoid and nonlymphoid cells.

MATERIALS AND METHODS

Cell Culture and Cloning. The human oligodendroglioma cell line ONS-21 was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 μg of kanamycin per ml, and 0.03% glutamine. For cell cloning, 20 ONS-21 cells were suspended in 10 ml of the above medium, and 100 μl of this suspension was plated into 96-well microtiter plates. The cultures were incubated for about 4 weeks, and cells were subsequently detached with 0.05% trypsin and 0.02% EDTA and replated for expansion. Three cell clones, designated ONS-21-B1, ONS-21-B2, and ONS-21-C2, were established.

Construction of the Expression Plasmids and Transfection to ONS-21-C2 Cells. Construction of the plasmids was carried out

Abbreviations: CNS, central nervous system; IL, interleukin; IL-2R, IL-2 receptor; IL-2Rα and IL-2Rβ, IL-2R α and β chains; MS, multiple sclerosis; EF, elongation factor; MBP, myelin basic protein; GalCer, galactosylcerebroside; NSE, neuron-specific enolase; GFAP, glial fibrillary acidic protein.

essentially following the general procedures (23). To construct pdKCRβ, the *Hha* I fragment containing the entire coding region of the human IL-2Rβ was inserted into *Bam*HI-cleaved pdKCR containing the simian virus 40 early promoter (24). The resulting plasmid, pdKCRβ, was introduced together with the neomycin-resistance gene (pSTneoB; ref. 25) into ONS21-C2 cells by the calcium phosphate precipitation method. Essentially the same cDNA fragment was inserted downstream of the promoter sequence for the human polypeptide-chain elongation factor-1α (EF-1α) gene (26) to construct pEFβ. Briefly, a 2.5-kilobase DNA fragment containing the promoter sequence, first exon and intron, and a part of the second exon (the initiator ATG is located further downstream of this DNA fragment) for the EF-1α gene was cut from pEF321CAT (gift of S. Nagata, Osaka Bioscience Institute, Osaka) by *Eco*RI and *Hin*dIII digestion. The human IL-2Rβ cDNA in pSP64 (Amersham) was excised by *Eco*RI and *Xba* I, whose cleavage sites are located within the vector DNA. The resulting promoter and cDNA fragments were ligated into a *Hin*dIII–*Xba* I backbone fragment of CDM8 (27) in which a *Hin*dIII linker had been inserted in lieu of the original cytomegalovirus promoter sequence after *Nru* I digestion of the vector DNA. The resulting plasmid, pEFβ, was then introduced together with pSTneoB into ONS-21-C2 cells by calcium phosphate precipitation. The neomycin-resistant clones were selected in the above described medium containing G418 (1 mg/ml), as described (28).

Assay for IL-2-Induced Cell Proliferation. The proliferative response of cells to IL-2 was monitored by [^{3}H]thymidine incorporation. Cells suspended in Dulbecco's modified Eagle's medium containing 1% fetal bovine serum were seeded into 96-well flat-bottom microtiter plates at a density of 3×10^3 cells per well. After a 24-hr incubation, various concentrations of recombinant IL-2 were added to those cultures, in the presence or absence of monoclonal antibodies against the human IL-2Rα [anti-Tac (29), 1:50 dilution of ascites fluid] or IL-2Rβ [Mik-β1 (14), 1:50 dilution of ascites fluid]. On day 3, cells were incubated with [^{3}H]thymidine [1 μCi (37 kBq) per well] for 18 hr (30).

General Procedures. DNA transfection, S1 nuclease mapping, Scatchard plot analysis, and IL-2 internalization assays were carried out following general procedures as described (12, 28).

RESULTS

Establishment of ONS-21 and Its Derivative Clones. The human oligodendroglioma cell line ONS-21 was established from an anaplastic (grade III) oligodendroglioma of a 48-year-old man by the primary explant technique (Fig. 1). Immunohistochemical analysis carried out after 22, 29, and 48 passages revealed that in all stages the cells were positive for myelin basic protein (MBP$^+$), galactocerebroside (GalCer$^+$), S-100 protein (S-100$^+$) and neuron-specific enolase (NSE$^+$) but negative for glial fibrillary acidic protein (GFAP$^-$) (results not shown). The cells at passages 22–29 were used to obtain several clones as described in *Materials and Methods*. Three of these clones, ONS-21-B1, ONS-21-B2, and ONS-21-C2, displayed essentially the same phenotype for the above markers as the parental ONS-21 cells (results not shown).

Expression of the Human IL-2Rβ cDNA. Although clones ONS-21-B1, ONS-21-B2, and ONS-21-C2 were all negative for the IL-2Rα and IL-2Rβ, the presence of very weakly staining cells with an anti-IL-2Rβ monoclonal antibody, Mik-β1, was observed in a population of the parental ONS-21 cells. Furthermore, expression of the IL-2Rβ mRNA was also detectable at very low level by S1 nuclease mapping analysis. On the other hand, similar analyses revealed no evidence for the expression of IL-2Rα (Y.O., unpublished observations). These results suggested that the IL-2R expressed in a certain population of ONS-21 cells at very low

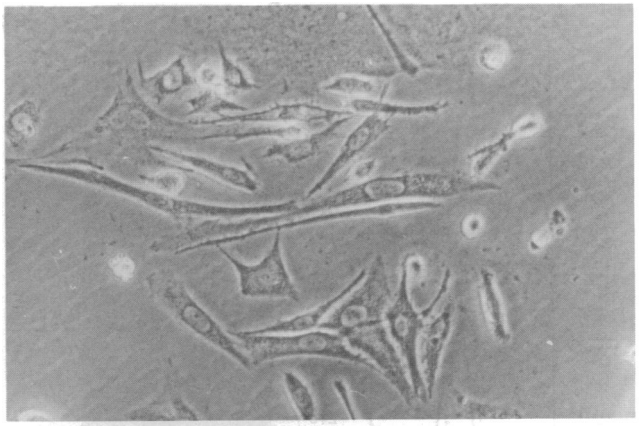

FIG. 1. Phase photomicrograph of human oligodendroglioma line ONS-21. The ONS-21 cells are adherent and morphologically distinct from lymphoid cells; they also differ from normal, mature oligodendrocytes with respect to the absence of typical processes. (×140.)

levels is similar or identical to the IL-2Rβ in lymphoid cells. Constitutive expression of IL-2Rβ but not IL-2Rα has been reported also in a certain population of lymphocytes (13–15, 31). Previously, cDNA expression studies have demonstrated that IL-2Rβ transduces the growth signal in lymphoid cells but does not function in fibroblasts when expressed singly or in combination with IL-2Rα (12, 32, 33, 36). However, the observations described above raised the possibility that the IL-2Rβ may be functional also in certain nonlymphoid cells such as ONS-21 oligodendroglioma cells.

To investigate the function of IL-2Rβ, the expression plasmid pdKCRβ was introduced into the cloned cell line ONS-21-C2 to amplify selectively the expression of IL-2Rβ. Two out of 11 G418-resistant clones, C2β-3 and C2β-6, expressed IL-2Rβ as judged by flow cytometry (Fig. 2*A*). S1 mapping analysis of the mRNA expressed in C2β-3 and C2β-6 revealed that IL-2Rβ-specific RNA was derived from the transfected cDNA but not from the endogenous IL-2Rβ gene (Fig. 2*B*), without affecting other phenotypic properties of the cells such as MBP$^+$, GalCer$^+$, and GFAP$^-$ (data not shown).

IL-2 Binding and Internalization in Clones C2β-3 and C2β-6. The IL-2 binding studies were performed with ^{125}I-labeled recombinant human IL-2. The following binding profiles were obtained by Scatchard analyses. Clones C2β-6 and C2β-3 displayed 1.7×10^3 and 1.3×10^3 IL-2 binding sites per cell with estimated K_d values of 2.2 and 1.7 nM, respectively (Fig. 3*A*). In contrast, IL-2 binding was undetectable in the parental ONS-21-C2 cells, as well as in the IL-2Rβ-negative, G418-resistant transformant clones. It has been shown that both intermediate- and high-affinity IL-2 receptors expressed in lymphoid cells can internalize the bound IL-2 (12). The IL-2Rβ expressed in clones C2β-6 and C2β-3 was also capable of internalizing bound IL-2 (Fig. 3*B*).

Proliferative Response of the IL-2Rβ-Expressing Cells to IL-2. Proliferative response of the IL-2Rβ-expressing clones to IL-2 was monitored by the [^{3}H]thymidine incorporation (Fig. 3*C*). IL-2 enhanced [^{3}H]thymidine incorporation by the C2β-6 and C2β-3 cells but not by the parental ONS-21-C2 cells at IL-2 concentrations above 1 nM. All other G418-resistant transformant clones that do not express IL-2Rβ did not show a proliferative response to IL-2. There was no indication for the induction of endogenous IL-2Rα and IL-2Rβ genes by IL-2 as judged by S1 mapping analysis of mRNA from IL-2-stimulated C2β-3 and C2β-6 cells (results not shown). Furthermore, proliferative responses to IL-2 detected in C2β-6 and C2β-3 cells were strongly inhibited by Mik-β1 but not by anti-Tac antibody (Table 1). Moreover, RNA blotting analysis showed no evidence for the expression

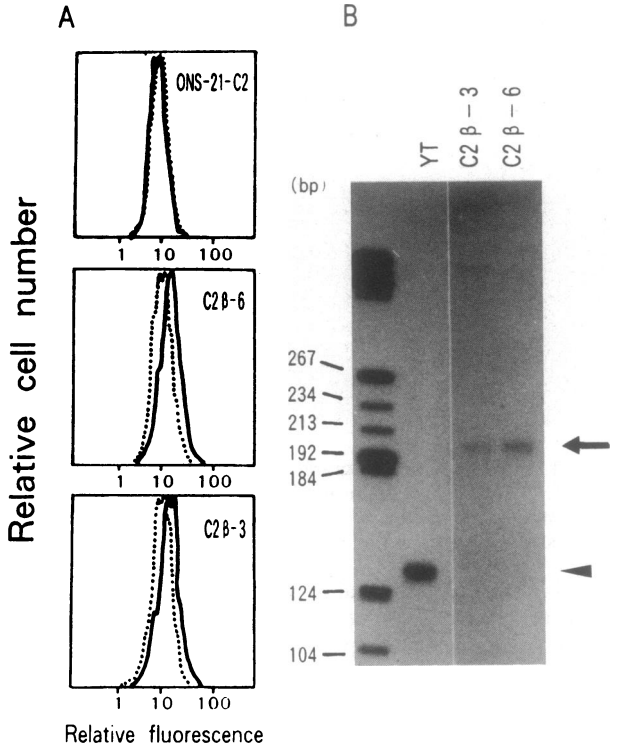

FIG. 2. (*A*) Expression of human IL-2Rβ cDNA on clones C2β-6 and C2β-3 and parental ONS-21-C2 cells. Both clones were stained with the monoclonal antibody to human IL-2Rβ, Mik-β1. Dotted line is a fluorescence profile of the cells stained with fluorescein-conjugated goat anti-mouse IgG alone. (*B*) S1 nuclease mapping analysis of the IL-2Rβ mRNA transcripts. A 565-base-pair (bp) *Eco*RI–*Pst* I DNA fragment (564 bp) containing 429 bp of simian virus 40 T-antigen early promoter region and 135 bp of human IL-2Rβ sequence was excised from pdKCRβ and cloned into *Eco*RI/*Pst* I-cleaved M13mp10 vector. Synthetic oligodeoxynucleotide (5′-ATGCCCAAGAGGTAGCC-3′) was labeled at the 5′ end and used as a primer (specific activity, 4.6×10^3 cpm/fmol). After the primer extension reaction, the probe DNA was incubated with 40 μg of total RNA from C2β-3 or C2β-6. Hybrid solution (10 μl) was treated with S1 nuclease (100 units) at 30°C for 40 min and analyzed by 6% PAGE (with 7 M urea). The mRNA from the endogenous gene should protect the probe DNA, 135 nucleotides long (arrowhead), whereas the cDNA-derived mRNA should protect DNA of 199 nucleotides (arrow). Lane at left contained size markers; YT is a natural killer-like human lymphoid cell line that expresses endogenous IL-2Rβ constitutively (37).

of IL-2Rα mRNA in these cells (data not shown). Hence, the IL-2 response in the C2β-6 and C2β-3 cells was most likely due to IL-2Rβ derived from the transfected cDNA.

The proliferative response of these transformants to IL-2 was not great, raising the question whether this is a reflection of IL-2Rβ density or the nature of the glial cells. To address this issue, an IL-2Rβ expression plasmid, pEFβ, in which expression of the cDNA was directed by a more potent promoter, the human EF-1α gene promoter (gift of S. Nagata), was constructed and introduced into ONS-21-C2. Although the exact number of expressed IL-2Rβ molecules is not available, the flow cytometry profiles clearly indicate that two stable transformant clones, C2β-101 and C2β-112, expressed IL-2Rβ molecules at much higher levels than C2β-3 and C2β-6 (cf. Figs. 4*A* and 2*A*). Similarly, the new transformants did not reveal the expression of IL-2Rα (data not shown). IL-2 markedly enhanced [^{3}H]thymidine incorporation by both clones (Fig. 4*B*). The response of C2β-101 and C2β-112 to IL-2 (10 nM) was much stronger (3- to 4-fold) than that of C2β-3 and C2β-6. These observations suggest that the proliferative response of the oligodendroglioma cells to IL-2 depends on the number of expressed IL-2Rβ molecules.

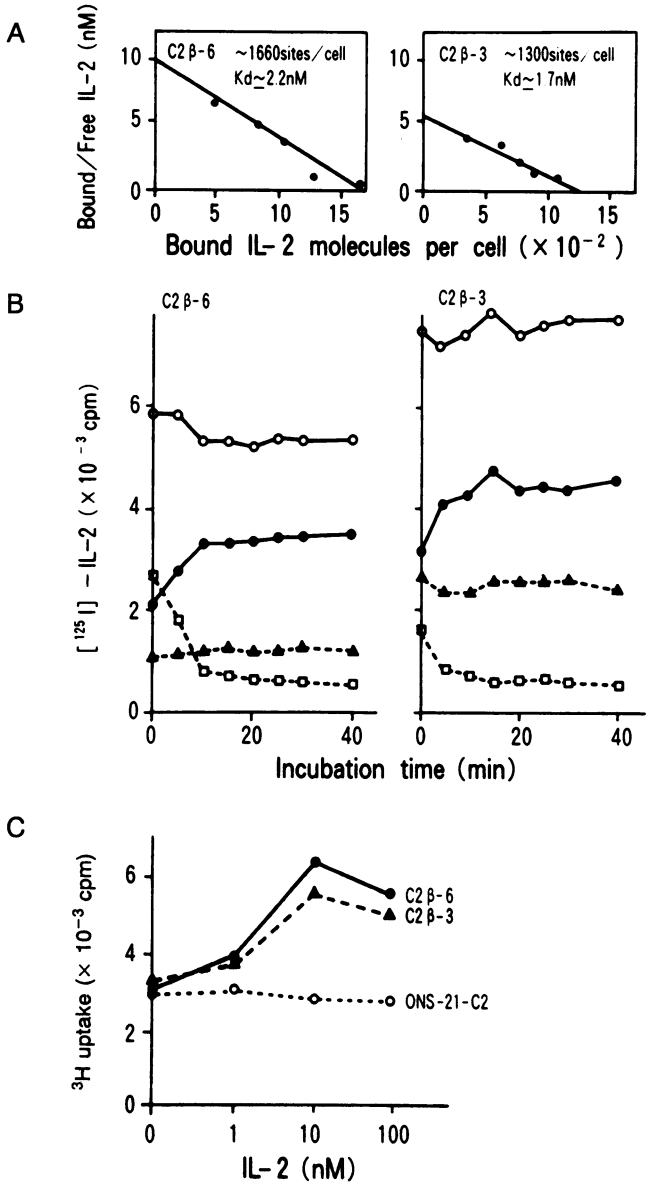

FIG. 3. (*A*) Scatchard analysis of ^{125}I-labeled IL-2 binding to clones C2β-6 and C2β-3. The number of IL-2 binding sites per cell and the receptor affinity (K_d) were determined by computer-assisted analysis of IL-2 binding data. (*B*) IL-2 internalization in clones C2β-6 and C2β-3. Cells (5×10^7) were treated with ^{125}I-labeled IL-2 (5 nM) at 4°C for 2 hr. The cells were suspended in the culture medium (37°C) and the kinetics of IL-2 internalization were examined. At each time point, radioactivity in the cell supernatant was measured (▲). The cells were then resuspended in pH 4 buffer and centrifuged through a layer of oil (12). The radioactivity in the cell pellet was measured to determine the level of pH 4-resistant, internalized IL-2 (●). The radioactivity in the supernatant was measured to determine the amount of cell surface-bound IL-2 dissociated in the pH 4 buffer (□). The sum of the radioactivities found in all three fractions is also graphed (○). (*C*) Proliferative responses of clones C2β-6 and C2β-3 and parental ONS-21-C2 to IL-2. Responses were monitored by [^{3}H]thymidine incorporation at various concentrations of recombinant human IL-2. The experiment was repeated several times, leading to the same conclusions.

DISCUSSION

Ample evidence has been provided that cytokines (lymphokines and monokines), which were identified originally as immunoregulatory mediators, function also in cells of the CNS (2, 4). However, very little is known at present about the nature and function of the receptors in cytokine-mediated signal transduction in the CNS.

Table 1. Effects of anti-IL-2R antibodies on the IL-2-induced proliferation of clones C2β-3 and C2β-6

Cells	Addition(s)	[^{3}H]thymidine incorporation, cpm	
		Experiment 1	Experiment 2
C2β-3	None	3222 ± 170	3236 ± 140
	IL-2	6248 ± 164	7066 ± 194
	IL-2 + anti-Tac	6060 ± 695	ND
	IL-2 + Mik-β1	2982 ± 819	3001 ± 128
	IL-2 + 3D-7	ND	6618 ± 106
C2β-6	None	3529 ± 75	4234 ± 80
	IL-2	7750 ± 514	7287 ± 309
	IL-2 + anti-Tac	7489 ± 688	ND
	IL-2 + Mik-β1	4152 ± 311	4747 ± 215
	IL-2 + 3D-7	ND	6788 ± 439

Cells were cultured for 72 hr with or without recombinant human IL-2 (10 nM) in the presence or absence of Mik-β1 (anti-IL-2Rβ), anti-Tac (anti-IL-2Rα), or control antibody, 3D-7 (an IgG2a monoclonal antibody). Results represent mean ± SD of triplicate samples. ND, not done.

The present study demonstrated that the cDNA-directed human IL-2Rβ in the human oligodendroglioma cell line ONS-21-C2, unlike IL-2Rβ expressed in fibroblast cell lines, behaves similarly to the IL-2Rβ in lymphoid cells; it binds IL-2 with intermediate affinity (K_d, ≈2 nM), internalizes IL-2, and transduces the growth signal(s). These findings thus give us further insight on the function of the IL-2R, by demonstrating that the same receptor for IL-2 can function in both lymphocytes and cells of CNS origin. The results also demonstrate that IL-2Rβ *per se* can transduce the IL-2-induced growth signal in the absence of IL-2Rα (Table 1). In the parental ONS-21 cells, IL-2Rβ expression was detectable, albeit at very low levels, by flow cytometry. However, IL-2 had no effect on the parental ONS-21 line (result not shown), presumably due to the low density of receptors. In fact, our cDNA expression studies indicate that the magnitude of the proliferative response of ONS-21-C2 depends on the density of IL-2Rβ (Figs. 3 and 4).

The above observations contrast with the results of IL-2Rβ cDNA expression studies in fibroblast lines, wherein the expressed IL-2Rβ cannot bind IL-2 and is therefore totally nonfunctional (12, 32, 33, 36). Collectively, these observations suggest the presence of a complementary component(s) that regulates the function of the receptor in both lymphoid cells and oligodendrocytes but not in fibroblasts. By means of crosslinking studies with radiolabeled IL-2, Merrill (34) found two classes of IL-2-binding proteins, 35 and 65 kDa, in human glioma cells. Furthermore, three other complex sizes (30, 45, and 75 kDa) appeared after incubation of the cells with IL-2 (34). It is possible that some of these may be the IL-2Rβ-associated molecule(s). Further studies are required to identify such associated molecule(s).

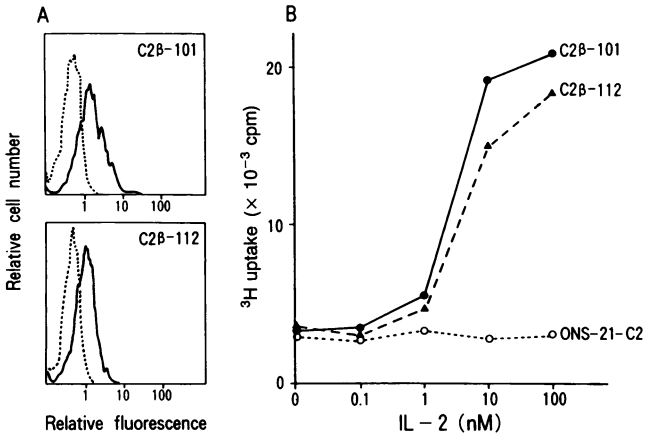

FIG. 4. (*A*) Expression of human IL-2Rβ cDNA on clones C2β-101 and C2β-112. These cells were stained with the monoclonal antibody to human IL-2Rβ, Mik-β1. Dotted line is a fluorescence profile of the cells stained with fluorescein-conjugated goat anti-mouse IgG alone. (*B*) Proliferative responses of clones C2β-101 and C2β-112, and parental ONS-21-C2 to IL-2, as monitored by [^{3}H]thymidine uptake at various concentrations of recombinant human IL-2.

It will be interesting to examine whether or not the IL-2Rβ-expressing transformant clones derived from ONS-21-C2 cells can be made to IL-2 at low concentrations by coexpression of IL-2Rα cDNA. Induction of the IL-2Rα by IL-2 via the IL-2Rβ has been documented in T cells as well as in IL-2-responsive, GalCer$^+$ glioblastoma cell lines (17); however, we have not detected IL-2Rα induction by IL-2 in either the parental ONS-21 cells or the IL-2Rβ-expressing transformants. Nor have we seen significant induction of MBP by IL-2, which has been reported previously in neonatal rat oligodendrocytes (35). In the GalCer$^+$ human glioblastoma cell lines, induction of MBP by IL-2 has not been observed (17). Presumably, such discrepancies are due to differences in the cell types.

While it is difficult to extrapolate from the present observations with transformed cells (i.e., oligodendroglioma) to what actually is occurring in normal oligodendrocytes, our findings indicate that IL-2-mediated growth in oligodendrocytes as reported previously (16, 17) may occur by a similar or identical mechanism, as described here. In pathological lesions of MS containing very few lymphoid cells, staining with a monoclonal antibody to IL-2 has been observed (22), suggesting that IL-2 may indeed be produced in the CNS. It is possible that IL-2 may stimulate oligodendrocytes in proliferation and remyelination under certain circumstances *in vivo*. Further investigation of the IL-2 system operating in neural cells may be of value in understanding the mechanism involved in the control of the CNS, including immune-mediated diseases such as MS.

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