Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4940–4944, May 1994
Immunology

The interleukin (IL) 2 receptor β chain is shared by IL-2 and a cytokine, provisionally designated IL-T, that stimulates T-cell proliferation and the induction of lymphokine-activated killer cells

RICHARD N. BAMFORD*, ANGUS J. GRANT†, JACK D. BURTON*, CHRISTIAN PETERS*, GLORIA KURYS*, CAROLYN K. GOLDMAN*, JENNIFER BRENNAN*, ERICH ROESSLER*, AND THOMAS A. WALDMANN*‡

*Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and †Laboratory for Cellular Immunology, Division of Cellular and Gene Therapy, Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD 20852

Contributed by Thomas A. Waldmann, February 22, 1994

ABSTRACT **Late-phase human T-cell lymphotropic virus I-associated adult T-cell leukemia cells express IL-2 receptors (IL-2R) but no longer produce IL-2. We have reported that the IL-2-independent adult T-cell leukemia line HuT-102 secretes a cytokine, provisionally designated IL-T, that stimulates T-cell proliferation and lymphokine-activated killer cell activity. Stimulation of proliferation of the cytokine-dependent human T-cell line Kit-225 mediated by HuT-102-conditioned medium or by 3200-fold-purified IL-T was not blocked by the addition of antibodies against IL-2 or IL-2R α subunit. However, IL-T-mediated stimulation of this human T-cell line was inhibited by addition of Mik-β1, an antibody that binds specifically to IL-2R β subunit. In addition, the activation of large granular lymphocytes to lymphokine-activated killer cells mediated by IL-T-containing conditioned medium was not blocked by antibodies directed toward IL-2 or IL-2α but was inhibited by an antibody to IL-2Rβ, suggesting the requirement of this receptor subunit for IL-T action. This conclusion was confirmed using an IL-3-dependent murine myeloid precursor cell line, 32D, that expresses IL-2Rα and IL-2Rγ, but not IL-2Rβ. Neither IL-2 nor IL-T stimulated 32D cell proliferation. However, after transfection with the gene encoding human IL-2Rβ, 32Dβ cells proliferated on addition of either cytokine. The IL-T-mediated stimulation of 32Dβ proliferation was inhibited by an anti-IL-2Rβ antibody but not by an anti-IL-2 antibody. Thus, the IL-T-mediated stimulation of T-cell and lymphokine-activated killer cell activation requires the expression of the IL-2Rβ subunit.**

T cells mediate important regulatory functions such as help and suppression and effector functions including the production of lymphokines and the cytotoxic destruction of antigen-bearing cells. Successful T-cell-mediated immune responses require that these cells change from a resting to an activated state. T-cell activation is initiated when an appropriately processed and presented antigen interacts with a heterodimeric T-cell receptor for that specific antigen. After this encounter, T cells enter a program of activation, leading to the *de novo* synthesis and secretion of cytokines including IL-2 and the induction of IL-2 receptor (IL-2R) expression (1, 2). The high-affinity IL-2R utilizes three independently regulated subunits: the 55-kDa IL-2Rα, the 70/75-kDa IL-2Rβ, and the 64-kDa IL-2Rγ (2–6). IL-2Rβ and IL-2Rγ are members of a large hematopoietin or cytokine receptor superfamily (6, 7). This superfamily includes receptors for such cytokines as IL-2 (β and γ receptor chains), IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, granulocyte/macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor, erythropoietin, and leukemia inhibitory factor (LIF) (7). The ILs that interact with this receptor superfamily manifest a high degree of pleiotropy and redundancy. The redundancy is explained in part by the sharing of common receptor subunits among members of the cytokine receptor superfamily. For example, IL-6, LIF, oncostatin-M, ciliary neurotrophic factor, and IL-11 utilize a public gp130 signaling subunit; whereas IL-3, IL-5, and GM-CSF utilize a common β_c subunit (8–11) in addition to their private ligand-specific receptors. There is a similar sharing of receptor elements within the IL-2R system (12–14). This receptor subunit sharing could have been anticipated from the observation that mice made deficient in IL-2 by homologous recombination develop normally during the first few weeks of life, yet IL-2R γ chain mutation results in X chromosome-linked severe combined immunodeficiency disease in humans (15, 16). It was subsequently demonstrated that the IL-2Rγ is a component required not only for high- and intermediate-affinity receptors for IL-2 but also for the actions of IL-4 and IL-7 and, possibly, IL-9 and IL-13 (12–14).

Disorders of the IL-2/IL-2R system are observed in human T-cell lymphotropic virus I-induced adult T-cell leukemia (ATL). In its early phases, ATL is associated with an autocrine process involving the coordinate expression of IL-2 and IL-2R (17). However, late-phase ATL and cell lines established from patients during this phase of their disease no longer produce IL-2 yet continue to express high-affinity IL-2Rs (18). We report elsewhere (19) that the IL-2-independent ATL line HuT-102 produces an ≈14-kDa cytokine, designated IL-T, that stimulates T-cell proliferation and lymphokine-activated killer (LAK) cell activity. In the present report, we indicate that the IL-T-mediated stimulation of cytokine-dependent T-cell lines or the activation of large granular lymphocytes (LGLs) is not blocked by addition of antibodies that bind specifically to IL-2 or to IL-2Rα yet is inhibited by the addition of Mik-β1, an antibody directed toward IL-2Rβ, suggesting the requirement of this receptor subunit for IL-T action. Furthermore, we confirmed the requirement of IL-2Rβ for IL-T action by using an IL-3-dependent cell line, 32D, that was examined prior to and after transfection with a gene encoding human IL-2Rβ. Thus the IL-T-mediated stimulation of the proliferation of cytokine-dependent T-cell lines and its induction of LAK cells when added to LGLs requires expression of IL-2Rβ that is shared by IL-2 and IL-T, thus expanding the spectrum of the promiscuity of the IL-2R system.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ATL, adult T-cell leukemia; CM, conditioned medium; GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; IL-2R, IL-2 receptor; LAK, lymphokine-activated killer; LIF, leukemia inhibitory factor; LGL, large granular lymphocyte; mAb, monoclonal antibody.

‡To whom reprint requests should be addressed at: Metabolism Branch, National Cancer Institute, National Institutes of Health, Building 10, Room 4N115, Bethesda, MD 20892.

MATERIALS AND METHODS

Monoclonal Antibodies (mAbs). Mik-β1 and anti-Tac that interrupt IL-2 binding to IL-2Rβ and IL-2Rα, respectively, and 7G7/B6, a nonblocking antibody specific for IL-2Rα, were prepared as described (3, 20, 21). A polyclonal antibody that neutralizes IL-2 action was obtained from Hoffmann-La Roche.

Cells. Kit-225, a human leukemic mature T-cell line that manifests high-affinity IL-2Rs involving IL-2Rα, IL-2Rβ, and IL-2Rγ subunits, was obtained from T. Uchiyama (Kyoto University, Kyoto). 32D, a murine myeloid precursor IL-3-dependent cell line that expresses IL-2Rα and IL-2Rγ but not IL-2Rβ, was a gift from Jacalyn H. Pierce (National Institutes of Health). LGLs depleted of T cells, B cells, and monocytes were prepared as described (22, 23). To derive 32Dβ cells that express the human IL-2Rβ chain, an extrachromosomal DNA expression vector, pREP9 (Invitrogen) encoding human IL-2Rβ was stably transfected into 32D cells by electroporation. Selection was initiated 24 hr after transfection by using G418 (1 mg/ml) in 10% (vol/vol) WEHI-3B conditioned medium (CM) containing IL-3. Wells containing a single colony were expanded and screened by flow cytometry analysis for IL-2Rβ expression. The 32Dβ cells were maintained in IL-2 (40 units/ml) rather than WEHI-3B CM to retain IL-2Rβ expression in the transfected cell line.

IL-2 and IL-T. *Escherichia coli*-produced recombinant human IL-2 was obtained from Hoffmann-La Roche. In functional studies of IL-T, both HuT-102 CM and 3200-fold-purified IL-T were used. CM was obtained from the uninduced HuT-102 cell line, a human T-cell lymphotropic virus I-associated ATL cell line that does not produce IL-2 but secretes IL-T (19). A 3200-fold-purified IL-T preparation was produced by the multistep procedure as outlined (19). The IL-T concentrations are expressed in units, where a single unit is defined as the quantity of IL-T required to achieve half-maximal proliferative stimulation when added to the cytokine-dependent CTLL-2 cell line. The biological activity in individual samples was quantitated by comparison to a dose-response curve obtained using an internal IL-T standard preparation.

Functional Assays. Cytotoxicity (^{51}Cr release) and proliferation ([^{3}H]thymidine incorporation) assays were performed as described (22, 23).

RESULTS

IL-T-Induced Stimulation of Proliferation of the Cytokine-Dependent Human T-Cell Line Kit-225 Is Blocked by an Antibody to IL-2Rβ. We have defined (19) a cytokine, provisionally designated IL-T, that stimulates T-cell and LAK-cell activity. In particular, the addition of CM from HuT-102, the human T-cell lymphotropic virus I-associated IL-2-independent ATL cell line, stimulated the proliferation of an IL-2-dependent CTLL-2 mouse indicator cell line (19). This stimulation of CTLL-2 cell proliferation mediated by the HuT-102 CM was not inhibited by the addition of an antiserum specific for IL-2. Furthermore, the uninduced HuT-102 cell line did not produce mRNA encoding IL-2 as assessed by Northern blot analysis and produced only barely detectable quantities of IL-2 mRNA as assessed by reverse transcription-PCR analysis, thus differentiating IL-T from IL-2. The present studies were directed toward determining whether the IL-2R α or β chains are shared by IL-2 and IL-T. In the initial studies, we defined the functional effects of mAbs on the IL-2- and IL-T-induced proliferation of the cytokine-dependent human T-cell line Kit-225 (subclone K6). To assess the functional effects of mAbs on proliferation mediated by IL-2 or IL-T through the IL-2Rs expressed on T cells, Kit-225-K6 cells were washed free of IL-2, rested for 24 hr without cytokine, and then cultured for an additional 53 hr with the cytokine being assayed. [^{3}H]Thymidine was added for the final 6 hr of culture. Rested cytokine-dependent Kit-225-K6 cells manifested a sigmoid proliferation dose-response curve on addition of IL-2. When mAbs (10 μg/ml) directed toward different epitopes expressed on IL-2Rα or IL-2Rβ were added to the cultures, distinct patterns of inhibition of cytokine-mediated proliferation were observed (Table 1). Addition of anti-Tac, which blocks IL-2 binding to IL-2Rα, was associated with a profound yet incomplete inhibition of IL-2-mediated proliferation of Kit-225-K6 cells. Addition of 7G7/B6, which reacts with a non-IL-2-binding portion of IL-2Rα, had no effect. Addition of mAb Mik-β1, which at 4°C abolishes high-affinity IL-2 binding to these cells, did not inhibit IL-2-induced proliferation when assessed at 37°C, as reported (20, 24-26). Although mAb Mik-β1 had no effect when used alone, it significantly augmented the anti-Tac-mediated inhibition of IL-2-induced proliferation. IL-12 also induced proliferation of Kit-225 cells that was not inhibited by addition of antibodies to IL-2, IL-2Rα, or IL-2 Rβ (Table 1).

A distinct pattern of mAb-mediated inhibition of proliferation was observed on addition of HuT-102 CM or 3200-fold-purified IL-T to Kit-225-K6 cells (Table 1). Specifically, IL-T-induced proliferation of Kit-225 cells was not inhibited by the addition of a neutralizing anti-IL-2 antiserum. Furthermore, no inhibition of IL-T-induced proliferation was observed on addition of anti-Tac or 7G7/B6, the antibodies directed toward IL-2Rα. However, in contrast to the observation with IL-2, addition of Mik-β1, the mAb directed toward IL-2Rβ, inhibited IL-T-mediated proliferation (Table 1). The inhibition mediated by Mik-β1 was not augmented by the further addition of anti-Tac. The results of these studies suggest that the IL-T-mediated stimulation of human T-cell proliferation requires the expression of the IL-2Rβ subunit.

Effects of mAbs on Cytokine-Induced Cytolytic Activity of IL-2Rβ-Expressing LGLs. Human LGLs express IL-2Rβ and IL-2Rγ and can be induced to become LAK cells by addition of IL-2 or IL-T (19, 27). To assess the effects of mAbs on IL-2- and IL-T-induced cytotoxicity, purified peripheral blood human LGLs were incubated for 36 hr with or without IL-2 or IL-T and mAbs before a final 4-hr coculture with ^{51}Cr-labeled Daudi cells. Addition of an antibody to IL-2 blocked IL-2-induced cytotoxicity (Table 2). Addition of

Table 1. Effect of mAbs on cytokine-induced proliferation of Kit-225 cells

	[^{3}H]Thymidine incorporated, cpm				
Cytokine	− mAb	+ anti-IL-2	+ anti-Tac	+ Mik-β1	+ Mik-β1 and anti-Tac
IL-2 (25 units)	165,800 ± 2540	150 ± 10	48,910 ± 4570	170,400 ± 8130	2,040 ± 80
IL-T (40 units)	38,040 ± 650	38,750 ± 1540	34,290 ± 330	12,682 ± 210	11,550 ± 240
IL-12 (2 ng/ml)	5,880 ± 110	6,580 ± 120	5,830 ± 190	5,630 ± 300	7,650 ± 190

Data are expressed as cpm observed less cpm in medium alone. Data are from Kit-225-K6 cells at 15,000 cells per well that had been rested without cytokine for 24 hr before the addition of cytokine and mAb. The cells were then incubated for an additional 48 hr at which time they were pulse-labeled with 1 μCi of [^{3}H]thymidine. Six hours later cells were harvested, and the radioactivity incorporated was determined. Ten micrograms of mAb was added when one mAb was used, whereas 5 μg of each mAb was used when Mik-β1 plus anti-Tac were added. Values shown represent the mean ± SEM of triplicate samples. The 3200-fold-purified IL-T preparation derived from HuT-102 CM was used.

Table 2. Effect of mAbs on cytokine-induced cytolytic activity mediated by LGL

	% specific lysis			
Cytokine	− mAb	+ anti-IL-2	+ anti-Tac	+ Mik-β1
IL-2 (10^{-10} M)	42	1	37	17
IL-2 (10^{-11} M)	37	1	27	10
IL-2 (10^{-12} M)	19	3	10	6
IL-T (1:10 CM)	37	36	36	6
IL-T (1:20 CM)	23	34	22	6
IL-T (1:40 CM)	17	15	15	6

Data are expressed as percent specific lysis from quadruplicates mediated by purified LGL in presence of cytokine or cytokine plus 20 μg of the mAb indicated. IL-T is given as the dilution of HuT-102 CM used.

anti-Tac did not have a major effect on IL-2-induced LGL killing of Daudi targets. However, addition of mAb Mik-β1 at 20 μg/ml to LGLs cultured with 10^{-10}, 10^{-11}, or 10^{-12} M IL-2 for 36 hr decreased the generation of IL-2-induced specific cytolytic activity against Daudi cells from 42%, 37%, and 19%, to 17%, 10%, and 6% lysis, respectively; that is, a reduction on mean from 33% specific lysis to 11% specific lysis on addition of Mik-β1 (Table 2). Thus, the addition of an antibody that blocks IL-2 binding to IL-2Rβ inhibited IL-2-mediated activation of LGLs that *ex vivo* express IL-2Rβ but not IL-2Rα.

A comparable pattern of inhibition on addition of Mik-β1 was observed when IL-T was used to activate LGLs (Table 2). Addition to purified LGLs of 1:10, 1:20, and 1:40 dilutions of HuT-102 CM containing IL-T led to the induction of 37%, 23%, and 17% specific lysis of Daudi targets, respectively. Addition of Mik-β1 (20 μg/ml) reduced this IL-T-mediated specific lysis to 6% in each case. In contrast to the observations with IL-2, there was no suppression of IL-T-induced cytotoxicity on addition of the antiserum to IL-2. Furthermore, the addition of anti-Tac did not affect the activation of LGLs mediated by IL-T-containing CM. From these studies on cytokine-induced LGL cytotoxic activity, we conclude that an antibody that blocks IL-2 binding to IL-2Rβ inhibits IL-T-mediated events, thereby supporting the view that IL-2Rβ but not IL-2Rα expression is required for IL-T action.

Confirmation of the Role of IL-2Rβ in IL-T-Mediated Signal Transduction by Using a Murine IL-3-Dependent Hematopoietic Cell Line After Transfection with a Gene Encoding Human IL-2Rβ. To further explore the role of the IL-2Rβ chain in IL-T-mediated functions, we examined the stimulation of proliferation mediated by IL-T on addition to the murine cell line 32D prior to and after transfection with a gene encoding human IL-2Rβ. 32D is an IL-3-dependent murine myeloid precursor cell line that expresses murine IL-2Rα and IL-2Rγ but not IL-2Rβ. The addition of IL-3 (5% WEHI-3B CM) to the parent 32D cell line-induced proliferation (Table 3). However, neither IL-2 nor IL-T (HuT-102 CM) stimulated the proliferation of the 32D cells that lack IL-2Rβ. When the 32D cell line was stably transfected with a gene encoding human IL-2Rβ and expression was confirmed by indirect flow cytometry analysis using mAb Mik-β1 and by Scatchard analysis of radiolabeled IL-2 binding (3000–5000 receptors per cell were demonstrated), the 32Dβ transfected line manifested a proliferative response to IL-2, HuT-102 CM, and the 3200-fold-enriched IL-T preparation (Table 3 and Fig. 1). The IL-2-mediated stimulation of proliferation of 32Dβ was inhibited by addition of the antiserum specific for IL-2. Furthermore, the addition of Mik-β1 (10 μg/ml) to 32Dβ cells blocked IL-2-induced proliferation. No inhibition was observed when a control antibody directed toward human IL-2Rα (nonreactive with murine 32Dβ cells) was utilized. As noted above, both HuT-102 CM and 3200-fold-purified IL-T stimulated the proliferation of 32Dβ cells (Fig. 1). This IL-T-mediated stimulation of 32Dβ was not abrogated by the addition of a polyclonal anti-IL-2 antiserum. In contrast, the IL-T-mediated stimulation of 32Dβ cells was inhibited by the addition of an antibody directed toward IL-2Rβ1 (Fig. 1). Thus our results support the view that the cytokine IL-T requires IL-2Rβ subunit expression for its stimulatory actions on T cells and LGLs.

Table 3. IL-2Rβ expression is required for IL-T-induced proliferation of 32D cells

	[^{3}H]Thymidine incorporated, cpm		
Cell line	IL-2 (50 units)	IL-T (20 units)	IL-3
32D	0	140 ± 10	173,200 ± 12,790
32Dβ	91,350 ± 2590	25,370 ± 480	130,500 ± 7,800

Data are expressed as cpm observed less cpm in medium alone. The cpm in medium alone was 700 cpm. The 3200-fold-purified IL-T preparation derived from HuT-102 CM was used. The 5% WEHI-3B CM supernatant was used to provide IL-3. The 32D hematopoietic cell line expresses murine IL-2Rα and IL-2Rγ but not IL-2Rβ. The 32Dβ cells are 32D cells transfected with the gene encoding human IL-2Rβ.

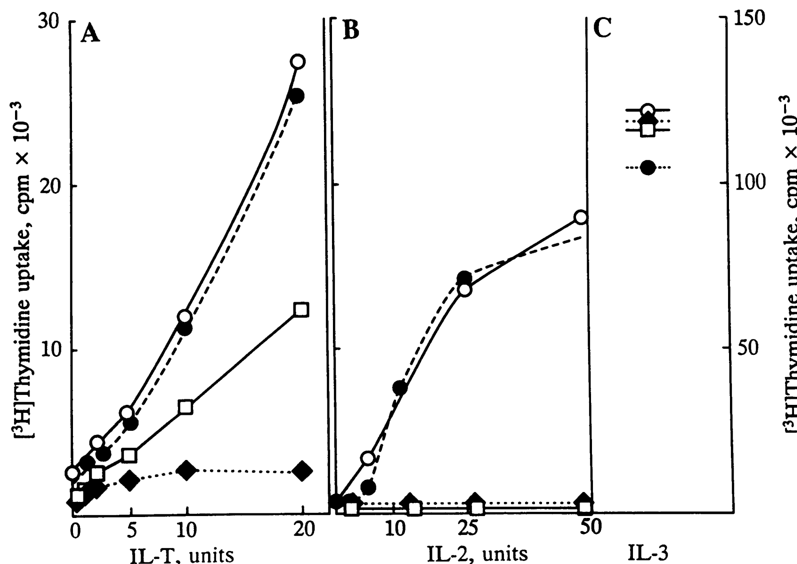

FIG. 1. Effect of antibody on the proliferative response of 32Dβ cells stimulated with 3200-fold-purified IL-T (*A*), IL-2 (*B*), or 5% WEHI-3B CM containing IL-3 (*C*). 32Dβ cells that express human IL-2Rβ were washed free of cytokine and dispensed in a 96-well microtiter plate at 15,000 cells per well. Ten micrograms of anti-Tac (●), Mik-β1 (◆), antiserum to IL-2 (□), or no antibody (○) was then added. One hour later the cytokines were added at the concentration indicated for 30 hr. [^{3}H]Thymidine was added at 24 hr of culture. The cells were harvested 6 hr later and the radioactivity incorporated was determined.

DISCUSSION

High-affinity IL-2 binding requires the interaction of IL-2Rα, IL-2Rβ, and IL-2Rγ (2, 4–6). IL-2Rβ and IL-2Rγ are members of the cytokine receptor superfamily, which includes receptors for IL-3, -4, -5, -6, -7, and -9 and for GM-CSF, granulocyte colony-stimulating factor, LIF, erythropoietin, and growth hormone (6, 7). Sharing common receptor subunits among related members of the cytokine receptor superfamily has been well documented with IL-6, IL-11, LIF, oncostatin-M, and ciliary neutrotrophic factor that share the signal transducer gp130 and with IL-3, IL-5, and GM-CSF that share a common β_c subunit (8–11). Such receptor subunit sharing is also a major feature of the IL-2R system with IL-2Rγ, a component not only of functional high- and intermediate-affinity IL-2Rs but also of receptors for IL-4 and IL-7 (12–14). In the present report, we extend the spectrum of IL-2R subunit sharing by reporting that IL-2Rβ is utilized by IL-2 and by a cytokine, provisionally designated IL-T, that stimulates T-cell and LAK-cell activities. The IL-T-mediated stimulation of the human cytokine-dependent Kit-225 cell line and activation of LGLs into LAK cells was not inhibited by anti-IL-2 antibodies but was inhibited by an antibody to IL-2Rβ, suggesting the requirement of this receptor subunit for IL-T action. This conclusion was confirmed using a murine IL-3-dependent myeloid precursor cell line, 32D, that expresses IL-2Rα and IL-2Rγ but not IL-2Rβ. Neither IL-2 nor IL-T stimulated 32D cell proliferation. However, after transfection with a gene encoding human IL-2Rβ, 32Dβ cells responded to both cytokines. Furthermore, IL-T-mediated stimulation of 32Dβ cells was inhibited by an antibody directed toward IL-2Rβ. Thus, IL-T represents a cytokine that stimulates T-cell and LAK-cell activation and requires IL-2Rβ subunit expression for these activities.

Most members of the cytokine superfamily of receptors are heteromers (8). Thus, it is probable that IL-T-mediated stimulation involves a receptor subunit in addition to IL-2Rβ. However, IL-T does not appear to require IL-2Rα for its action. This conclusion is based on the observation that IL-T induces activation of LGLs that express IL-2Rβ and IL-2Rγ but not IL-2Rα. Furthermore, in contrast to the situation with IL-2, IL-T-induced stimulation of the high-affinity IL-2R-expressing Kit-225-K6 cell line was not inhibited by addition of the anti-Tac mAb, which blocks the interaction of IL-2 with IL-2Rα and thereby inhibits IL-2-mediated proliferation. Thus, it is probable that a private ligand receptor chain exists that is specific for IL-T and that is not shared by IL-2.

Most cytokines are pleotrophic in their action, with cellular targets determined by the distribution of cell surface receptors specific for the cytokine. The IL-2R subunits are independently regulated and are expressed in different combinations, as might have been anticipated by the observations to date that IL-2Rα is used solely by IL-2, that IL-2Rγ is shared by IL-2, IL-4, and IL-7 and possibly by IL-9 and IL-13, whereas IL-2Rβ is required for the actions of both IL-2 and IL-T. Most resting T cells, B cells, LGLs, and monocytes do not express IL-2Rα but can be induced to express this receptor (2, 4). In contrast to this lack of IL-2Rα expression by most normal resting cells, IL-2Rγ is widely expressed. The IL-2Rβ subunit required for the action of not only IL-2 but also IL-T is also widely distributed on adult and fetal cells. IL-2Rβ is coexpressed with IL-2Rα on activated mononuclear cells that manifest the high-affinity IL-2R (2, 20). IL-2Rβ is also expressed by cells that do not bear IL-2Rα, including resting CD8-expressing cytotoxic T lymphocytes, select B lymphocytes, monocytes, LGLs, human neutrophils, γδ-T-cell-receptor-expressing thymocytes, as well as T-cell-receptor-ligation-activated CD4, CD8 double-positive thymocytes (24, 28–31). There is an increase in IL-2Rβ expression on CD4 and CD8 cells in association with acute allograft rejection, indicating that IL-2Rβ expression appears to increase on alloreactive T cells (32). IL-2Rβ is also expressed by fetal tissues, including embryonic fibroblasts (33). The *in utero* treatment with an anti-IL-2Rβ mAb completely abrogated the development of Thy-1-expressing dendritic epidermal cells (34). Finally, it has been reported that embryonic fibroblasts expressing IL-2Rβ but not IL-2Rγ respond to an IL-2 preparation (35). Thus, a broad array of cells display IL-2Rβ that is required for the action of IL-T.

One major clinical corollary that can be derived from the demonstration of sharing of cytokine receptor subunits is that one may obtain effects with receptor-directed therapy that differ from those observed with therapeutic agents that inhibit the synthesis of an individual cytokine. Such a difference in effect that is a consequence of receptor subunit sharing underlies the observation that mice made deficient in IL-2 by homologous recombination develop normally during the first 3 or 4 weeks of life, yet severe combined immunodeficiency is observed when there are mutations of IL-2Rγ that is shared by IL-2, IL-4, and IL-7 (12–14). The observation reported herein that IL-2Rβ not only is a component of high- and intermediate-affinity IL-2Rs but also is required for the action of IL-T suggests that IL-2Rβ-directed therapy may yield more profound immunosuppression than can be achieved by the inhibition of the synthesis of IL-2 alone.

Most of the published IL-2R-directed therapeutic approaches have focused on IL-2Rα (36, 37). Considerable success has been achieved with this approach when applied to the prevention of allograft rejection, the treatment of graft-vs.-host disease, or IL-2Rα-expressing leukemia/lymphoma (36–38). Nevertheless, there are certain functional characteristics of anti-IL-2Rα mAbs that when used alone may limit their immunosuppressive effect. Specifically, anti-IL-2Rα mAbs such as anti-Tac do not inhibit induction of cytotoxic LAK-cell activity mediated by IL-2, since the target, resting LGLs, do not express IL-2Rα but only IL-2Rβ and γ (24, 27). Furthermore, as discussed herein, IL-T-mediated functions are not reversed by antibodies directed to IL-2Rα.

To circumvent the limitations inherent in antibodies directed toward IL-2Rα, antibodies such as Mik-β1 that are directed toward IL-2Rβ were developed for therapeutic use (20, 39). In contrast to IL-2Rα, IL-2Rβ is constitutively expressed by LGLs (27), and the IL-2-enhanced cytotoxic activity of these cells is inhibited by Mik-β1 (24, 39). Furthermore, Mik-β1 acts synergistically with anti-Tac to profoundly inhibit IL-2-induced proliferation of activated T cells (20, 39). Finally, antibodies directed toward IL-2Rβ may be more effective in inhibiting the functional effects mediated by IL-T on high-affinity-IL-2R-expressing T cells than they are in inhibiting IL-2-induced activation of such cells. We base this conclusion on our observation that Mik-β1 was effective in inhibiting the IL-T-mediated proliferation of Kit-225-K6 cells that express high-affinity receptors, whereas it was relatively ineffective in inhibiting IL-2-mediated proliferation of such cells. On the basis of previous investigations involving IL-2 and the present studies on IL-T-mediated stimulation, it should be of value to define the therapeutic potential of a combination of anti-IL-2Rα (e.g., anti-Tac) and anti-IL-2Rβ (e.g., Mik-β1) mAbs to yield effective immunosuppression for therapy, since this combination of antibodies should inhibit activated T-cell and LGL functions induced by either IL-2 or IL-T.

In conclusion, IL-2Rβ is shared by the cytokines IL-2 and IL-T. Our developing understanding of the structure and function of the promiscuous multisubunit IL-2R on the surface of activated cells of the immune system and our capacity to produce humanized forms of mAbs directed toward the different subunits of the IL-2R provide the scientific basis for

the development of receptor-directed approaches for the prevention of allograft rejection and for treatment of graft-vs.-host disease, autoimmune disorders, and IL-2R-expressing leukemias and lymphomas.

1. Smith, K. A. (1980) *Immunol. Rev.* **51,** 337–357.
2. Waldmann, T. A. (1989) *Annu. Rev. Biochem.* **58,** 875–911.
3. Uchiyama, T., Broder, S. & Waldmann, T. A. (1981) *J. Immunol.* **126,** 1393–1397.
4. Waldmann, T. A. (1991) *J. Biol. Chem.* **266,** 2681–2684.
5. Tsudo, M., Kozak, R. W., Goldman, C. K. & Waldmann, T. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9694–9698.
6. Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. & Sugamura, K. (1992) *Science* **257,** 379–382.
7. Bazan, J. F. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6934–6938.
8. Kishimoto, T., Taga, T. & Akira, S. (1994) *Cell* **76,** 253–262.
9. Miyajima, A., Kitamura, T., Harada, N., Yokota, T. & Arai, K.-I. (1992) *Annu. Rev. Immunol.* **10,** 295–331.
10. Davis, S., Aldrich, T. H., Stahl, N., Pan, L., Taga, T., Kishimoto, T., Ip, N. Y. & Yancopoulos, G. D. (1993) *Science* **260,** 1805–1808.
11. Gearing, D. P., Comeau, M. R., Friend, D. J., Gimpel, S. D., Thut, C. J., McGourty, J., Brasher, K. K., King, J. A., Gillis, S., Mosley, B., Ziegler, S. F. & Cosman, D. (1992) *Science* **255,** 1434–1437.
12. Kondo, M., Takeshita, T., Ishii, N., Nakamura, M., Watanabe, S., Arai, K.-I. & Sugamura, K. (1993) *Science* **262,** 1874–1877.
13. Noguchi, M., Nakamura, Y., Russell, S. M., Ziegler, S. F., Tsang, M., Cao, X. & Leonard, W. L. (1993) *Science* **262,** 1877–1880.
14. Russell, S. M., Keegan, A. D., Harada, N., Nakamura, Y., Noguchi, M., Leland, P., Friedmann, M. C., Miyajima, A., Puri, R. K., Paul, W. I. & Leonard, W. J. (1993) *Science* **262,** 1880–1883.
15. Schorle, H., Holtschke, T., Hünig, T., Schimpl, A. & Horack, I. (1991) *Nature (London)* **352,** 621–624.
16. Noguchi, M., Yi, H., Rosenblatt, H. M., Filipovich, A. H., Adelstein, S., Modi, W. S., McBride, O. W. & Leonard, W. J. (1993) *Cell* **73,** 147–157.
17. Maeda, M., Arima, N., Daitoku, Y., Kashihara, M., Okamoto, H., Uchiyama, T., Shirono, K., Matsuoka, M., Hattori, T., Takatsuki, K., Ikuta, K., Shimizu, A., Honjo, T. & Yodoi, J. (1987) *Blood* **70,** 1407–1411.
18. Tendler, C. L., Greenberg, S. J., Burton, J. D., Danielpour, D., Kim, S.-J., Blattner, W. A., Manns, A. & Waldmann, T. A. (1991) *J. Cell. Biochem.* **46,** 302–311.
19. Burton, J. D., Bamford, R. N., Peters, C., Grant, A. J., Kurys, G., Goldman, C. K., Brennan, J., Roessler, E. & Waldmann, T. A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4935–4939.
20. Tsudo, M., Kitamura, F. & Miyasaka, M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1982–1986.
21. Rubin, L. A., Kurman, C. C., Biddison, W. E., Goldman, N. D. & Nelson, D. L. (1985) *Hybridoma* **4,** 91–102.
22. Grant, A. J., Merchant, R. E. & Hall, R. E. (1989) *Immunology* **66,** 117–124.
23. Phillips, J. H. & Lanier, L. L. (1986) *J. Exp. Med.* **164,** 814–825.
24. Grant, A. J., Roessler, E., Ju, G., Tsudo, M., Sugamura, K. & Waldmann, T. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2165–2169.
25. Kamio, M., Uchiyama, T., Arima, N., Itoh, K., Ishikawa, T., Hori, T. & Uchino, H. (1990) *Int. Immunol.* **2,** 521–530.
26. Saito, Y., Ogura, T., Kamio, M., Sabe, H., Uchiyama, T. & Honjo, T. (1990) *Int. Immunol.* **2,** 1167–1177.
27. Tsudo, M., Goldman, C. K., Bongiovanni, K. F., Chan, W. C., Winston, E. F., Yagita, M., Grimm, E. A. & Waldmann, T. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5394–5398.
28. Begley, C. G., Burton, J. D., Tsudo, M., Brownstein, B. H., Ambrus, J. L., Jr., & Waldmann, T. A. (1990) *Leuk. Res.* **14,** 263–271.
29. Espinoza-Delgado, I., Ortaldo, J. R., Winkler-Pickett, R., Sugamura, K., Varesio, L. & Longo, D. L. (1990) *J. Exp. Med.* **171,** 1821–1826.
30. Djeu, J. Y., Liu, J. H., Wei, S., Rui, H., Pearson, C. A., Leonard, W. J. & Blanchard, D. K. (1993) *J. Immunol.* **150,** 960–970.
31. Park, J.-H., Mitnacht, R., Torres-Nagel, N. & Hünig, T. (1993) *J. Exp. Med.* **177,** 541–546.
32. Niguma, T., Sakagami, K., Kawamura, T., Haisa, M., Fujiwara, T., Kusaka, S., Uda, M. & Orita, K. (1991) *Transplantation* **52,** 296–302.
33. Plaisance, S., Rubinstein, E., Alileche, A., Sahraoui, Y., Krief, P., Augery-Bourget, Y. A., Jasmin, C., Suarez, H. & Azzarone, B. (1992) *Int. Immunol.* **4,** 739–746.
34. Tanaka, T., Takenchi, Y., Shiohara, T., Kitamura, F., Nagasaka, Y., Hamamura, K., Yagita, H. & Miyasaka, M. (1991) *Int. Immunol.* **4,** 487–491.
35. Plaisance, S., Rubinstein, E., Alileche, A., Benoit, P., Jasmin, C. & Azzarone, B. (1993) *Int. Immunol.* **5,** 843–848.
36. Waldmann, T. A. (1989) *J. Natl. Cancer Inst.* **81,** 914–923.
37. Waldmann, T. A. (1992) *Annu. Rev. Immunol.* **10,** 675–704.
38. Waldmann, T. A., White, J. D., Goldman, C. K., Top, L., Grant, A., Bamford, R., Roessler, E., Horak, I., Zaknoen, S., Kasten-Sportes, C., England, R., Horak, E., Mishra, B., Dipre, M., Hale, P., Fleisher, T. A., Junghans, R. P., Jaffe, E. S. & Nelson, D. L. (1993) *Blood* **82,** 1701–1712.
39. Hakimi, J., Ha, V. C., Lin, P., Campbell, E., Gately, M. K., Tsudo, M., Payne, P. W., Waldmann, T. A., Grant, A. J., Tsien, W.-H. & Schneider, W. P. (1993) *J. Immunol.* **151,** 1075–1085.