IL-2 exerts multiple biological activities upon binding to its specific cell surface receptors. The IL-2 receptor (IL-2R) is composed of at least two subunits, the p55 (CD25/Tac) and the p75 glycoproteins. p55 and p75 both bind IL-2 independently with either low or intermediate affinity, respectively, whereas a heterodimeric receptor composed of p55 and p75 binds IL-2 with high affinity (1, 2). p75 but not p55 has been suggested to be responsible for signal transduction (1, 2).

Human PBL are readily activated by IL-2 to exhibit augmented cytotoxicity against NK-sensitive and NK-resistant target cells, which is known as the lymphokine-activated killer (LAK) phenomenon (3). CD3-CD16+ NK cells are the predominant LAK precursor and effector cells (4). Recently, Phillips et al. (5) have directly assessed the involvement of the p75 IL-2R in the activation of peripheral blood NK cells by using a recently developed mAb, termed TU27, which specifically blocks IL-2 binding to the p75 IL-2R (6).

Although TU27 mAb preferentially reacts with CD3-CD16+ NK cells (5), we have also demonstrated that peripheral blood CD8+ but not CD4+ T cells exhibit a substantial reactivity with TU27 mAb, as estimated by flow cytometry (7). In addition, several reports have described the direct activation of resting T cells by IL-2, and again the p75 IL-2R has been implicated in this response (8-10). In the present study we have examined the role of p75 and p55 IL-2R in rapidly inducing peripheral blood T cell cytolytic activity by using the mAbs abrogating IL-2 binding to these subunits.

### Materials and Methods

**Monoclonal Antibodies.** TU27 mAb reacts with the p75 IL-2R and blocks IL-2 binding to this subunit (6). H-31 mAb reacts with the p55 IL-2R and blocks IL-2 binding to this subunit (11). WT31 mAb reactive with the TCR composed of α/β chains (TCRα/β) was purchased from Sanbio (Uden, The Netherlands). TCRβ1 mAb reactive with the TCR δ chain (12) was kindly provided by Dr. M. Brenner (Dana Farber Cancer Institute, Boston, MA). The hybridoma cells producing anti-CD3 mAb (OKT3) and anti-CD8 mAb (OKT8) were obtained from the American Type Culture Collection (Rockville, MD). The hybridoma cell producing anti-nitrophenyl (NP) hapten mAb (C6-8-2) was kindly provided by Dr. T.
Azuma (Nagoya City University, Nagoya, Japan). These mAbs were purified from ascites by using Affi-Gel Protein A MAPSII kit (Bio-Rad Laboratories, Richmond, CA). Antibody heteroconjugate composed of Fab fragments of anti-CD3 and anti-NP mAbs (OKT3 × anti-NP) was prepared as described by Brennan et al. (13). FITC-conjugated anti-CD4 (Leu 3a), -CD8(Leu 2a), -CD16(Leu 11a), phycoerythrin (PE)-conjugated streptavidin, and unlabeled anti-CD3 (Leu 4) were purchased from Becton Dickinson (Mountain View, CA). FITC-conjugated anti-TCRβ chain (TCRβ1) was purchased from T Cell Sciences (Cambridge, MA).

**Cell Culture.** PBMC were isolated from the blood of healthy donors using Ficoll-Hypaque. After depletion of monocytes and B cells by adherence to plastic dishes and passage through nylon wool, nonadherent lymphocytes were fractionated on discontinuous gradients consisting of 30, 35, 40 and 47.5% Percoll (Pharmacia LKB Biotechnology, Tokyo, Japan), as described (14). The high buoyant density lymphocytes isolated from the interface between 40 and 47.5% contained ~95% CD3⁺CD16⁻ T cells, as estimated by flow cytometry. Cells (10⁶ cells/ml) were cultured for 18 h in RPMI 1640 medium supplemented with 10% FCS (Gibco Laboratories, Grand Island, NY), l-glutamine, and antibiotics in the presence or absence of human rIL-2 (generously provided by Shionogi Pharmaceutical Co., Osaka, Japan). Anti-IL-2R mAbs were added to the cultures 30 min before adding rIL-2.

**Cytotoxicity Assays.** Cytotoxicity was measured in a standard 4-h ⁵¹Cr-release assay. In all experiments, a graded number of effector cells were added to 10⁴ ⁵¹Cr-labeled target cells. In some experiments, target cells were modified with NP as described (15). Anti-TCR/CD3 mAbs and antibody heteroconjugate were added to the effector/target mix to a final concentration of 5 μg/ml.

**Immunofluorescence and Flow Cytometry.** Immunofluorescence and flow cytometric analysis were performed as described (16). Samples were analyzed on a FACScan (Becton Dickinson) and data were processed by using the Consort 30 program.

**Results and Discussion**

Cytotoxic T cells can be directed to lyse incompatible target cells in the presence of anti-TCR/CD3 mAbs either when the target cells bear receptor for Fc portion of the mAbs (17) or when the mAbs are crosslinked to an appropriate anti-target cell antibody (18). To examine potential cytolytic activities induced in peripheral blood T cells after brief exposure to IL-2 regardless of their target specificity, we used a heteroconjugate antibody composed of Fab fragments of anti-CD3 and anti-NP hapten mAbs (OKT3 × anti-NP), and cytotoxicity was tested against a NP-modified murine T lymphoma EL-4. As previously described by Perez et al. (19), peripheral blood T cell cytotoxicity assessed by this system could be rapidly augmented by exposure to IL-2 in a dose-dependent manner (Fig. 1A).

**Figure 1.** Effects of anti-IL-2R mAbs on IL-2-induced cytolytic activity in peripheral blood T cells. PBMC were cultured with various concentrations (A) or 100 U/ml (B) of rIL-2 in the presence or absence (O) of 10 μg/ml (A) or various concentrations (B) of H-31 (○), TU27 (▲), or both H-31 and TU27 (■) mAbs for 18 h. Then the cytotoxicity was tested against NP-modified EL-4 in the presence of OKT3 × anti-NP heteroconjugate at an E/T ratio of 5:1. Open triangle (B) indicates basal cytotoxicity after culture without IL-2. Cytotoxicity in the absence of heteroconjugate and that against unmodified EL-4 was negligible (<3%).
To estimate the involvement of p55 and p75 IL-2R in this rapid induction of cytotoxicity in circulating T cells, cytotoxicity was tested after culturing PBMC with rIL-2 in the presence of H-31 and/or TU27 mAbs. TU27 mAb dramatically inhibited the induction of peripheral blood T cell cytotoxicity in a broad range of IL-2 concentrations (Fig. 1A) and in a dose-dependent manner (Fig. 1B). Although H-31 mAb alone had little effect on the IL-2-induced cytotoxicity, the combination with TU27 inhibited the response more efficiently than did TU27 alone (Fig. 1A, B). Essentially identical results were obtained with PBMC isolated from five different blood donors regarding the effect of TU27; however, a small but significant inhibition was observed with H-31 alone in some cases, as shown in Fig. 1A.

We next examined the involvement of p55 and p75 IL-2R in inducing cytotoxicity in T cell subpopulations. Human PBL contain two distinct types of T cells bearing either TCR-α/β or TCR-γ/δ (19). Among TCR-α/β-bearing T (Tαβ) cells, CD8+ T cells include the precursors of classical cytotoxic T cells and have been reported to be responsible for the anti-CD3-targeted cytotoxicity in peripheral blood T cells (19). TCR-γ/δ-bearing T (Tγδ) cells in PBL predominantly lack CD4 and CD8 and exhibit MHC-unrestricted cytotoxicity after culturing with IL-2 (20). After culturing high buoyant density PBL (~95% CD3+CD16- T cells) with rIL-2, cytotoxicity was tested against a murine mastocytoma P815 bearing FcR for murine IgG1 in the presence of anti-CD3 (Leu4, IgG1), anti-TCR-α/β (WT31, IgG1), or anti-TCR-γ/δ (TCRδ-1, IgG1) mAbs. As shown in Fig. 2, substantial levels of cytotoxicity were induced in both Tαβ and Tγδ cells after exposure to IL-2. A prior depletion of CD8+ cells from high density T cells by treatment with OKT8 mAb and complement totally abrogated the IL-2–induced cytotoxicity in Tαβ cells but not in Tγδ cells (data not shown), indicating that the cytotoxic precursor in Tαβ cells are CD8+, as described previously (19). The IL-2 responses of both Tαβ and Tγδ cells, assessed in this system, were almost completely blocked by TU27 mAb and also partially blocked by H-31 mAb (Fig. 2). These results clearly indicate that the p75 IL-2R expressed on CD8+ Tαβ and Tγδ cells are predominantly responsible for direct activation of these cells by IL-2 and also demonstrate a minor but significant contribution of the p55 IL-2R.

The expression of functional IL-2R on peripheral blood T cell subpopulations, revealed as above, was confirmed by immunofluorescent staining with H-31 and TU27 mAbs in flow cytometric analysis (Fig. 3). As demonstrated previously (5, 7), CD16+ NK cells and CD8+ T cells express the p75 but not p55 IL-2R. TCR-δ1+ T cells express the p75 IL-2R at an intermediate level between those expressed on NK and CD8+ T cells. Essentially identical patterns of p75 expression were consistently ob-
served with \( \gamma \delta \) cells from five donors, although \( \gamma \delta \) cells from some donors contained a subpopulation expressing also very low levels of the p55 IL-2R as represented in Fig. 3. Recently, Aribia et al. have demonstrated a similar level of p55 and p75 IL-2R expression in circulating NK and T cells, as estimated by \(^{125}\)I-labeled IL-2 binding (10). The very low level of high affinity receptor expression in peripheral blood T cells described by them may be responsible for the minor contribution of p55 IL-2R observed in Fig. 2, although it was hardly detectable on CD8\(^+\) T cells in our flow cytometric analysis. In summary, our present results directly indicate that the p75 IL-2R is predominantly responsible for direct activation of peripheral blood T cells by IL-2 and is functional in inducing cytotoxic activity in CD8\(^+\) T\(\alpha\)\(\beta\) and \(\gamma\delta\) cells as well as in NK cells.

**Summary**

By using mAb and flow cytometry, a constitutive expression of the p75 IL-2R was revealed in human peripheral blood CD8\(^+\) T cells and TCR\(\delta\)-1\(^+\) T cells as well as in CD16\(^+\) NK cells. Anti-p75 IL-2R mAb almost completely inhibited the induction of cytolytic activity in these T cells by brief exposure to IL-2, as estimated by anti-TCR/CD3 mAb–targeted cytotoxicity. While anti-p55 IL-2R mAb alone inhibited the response only modestly, maximal inhibition was achieved by combining...
both anti-p55 and anti-p75 IL-2R mAbs. These results indicate that the p75 IL-2R constitutively expressed on peripheral blood CD8+ T cells and TCRβ-1+ T cells is predominantly responsible for the direct activation of these cells by IL-2.

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