Brief Definitive Report

ACTIVATION OF PERIPHERAL BLOOD T CELLS VIA THE p75 INTERLEUKIN 2 RECEPTOR

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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 (TCR-α/β) was purchased from Sanbio (Uden, The Netherlands). TCRδ-1 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.

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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-TCRδ chain (TCRδ-1) 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 (10^6 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 ^{51}Cr-release assay. In all experiments, a graded number of effector cells were added to 10^4 ^{51}Cr-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. 1 *A*).

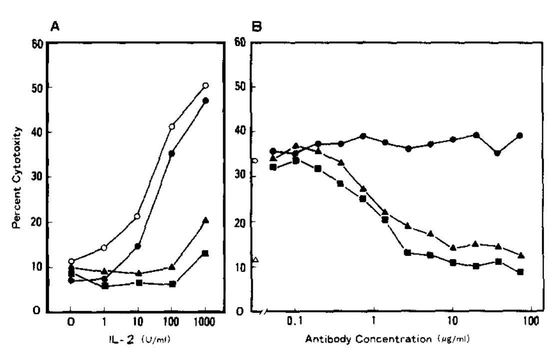

FIGURE 1. Effects of anti-IL-2R mAbs on IL-2-induced cytolytic activity in peripheral blood T cells. PBMC were cultured with various concentrations (*A*) or 100 U/ml (*B*) of rIL-2 in the presence or absence (○) of 10 μg/ml (*A*) or various concentrations (*B*) of H-31 (●), TU27 (▲), or both H-31 and 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. 1 *A*) and in a dose-dependent manner (Fig. 1 *B*). 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. 1 *A, 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. 1 *A*.

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 (T$\alpha\beta$) 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 (T$\gamma\delta$) 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 T$\alpha\beta$ and T$\gamma\delta$ 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 T$\alpha\beta$ cells but not in T$\gamma\delta$ cells (data not shown), indicating that the cytotoxic precursor in T$\alpha\beta$ cells are $CD8^+$, as described previously (19). The IL-2 responses of both T$\alpha\beta$ and T$\gamma\delta$ 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 $CD8^+$ T$\alpha\beta$ and T$\gamma\delta$ 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-δ-1^+ 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-

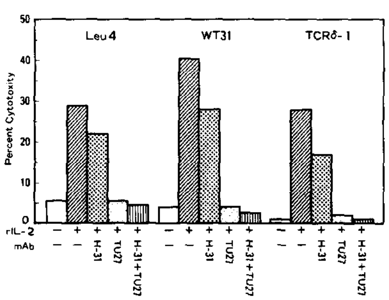

FIGURE 2. Effects of anti-IL-2R mAbs on IL-2-induced cytolytic activities in TCR-α/β- or TCR-γ/δ-bearing T cells. High buoyant density PBL were cultured with or without (□) 100 U/ml of rIL-2 in the presence or absence (▨) of H-31 (▩), TU27 (■), or the both H-31 and TU27 (▥) mAbs (10 μg/ml each). After 18-h cultures, the cells were harvested and their cytolytic ability was tested against a murine mastocytoma P815 bearing FcR for murine IgG1 in the presence or absence of anti-CD3(Leu 4, IgG1), anti-TCR-α/β (WT31, IgG1), or anti-TCR-γ/δ (TCRδ-1, IgG1) mAbs, as indicated, at an E/T ratio of 20:1. Basal cytotoxity against P815 in the absence of anti-TCR/CD3 mAbs were negligible (<5%) in all samples.

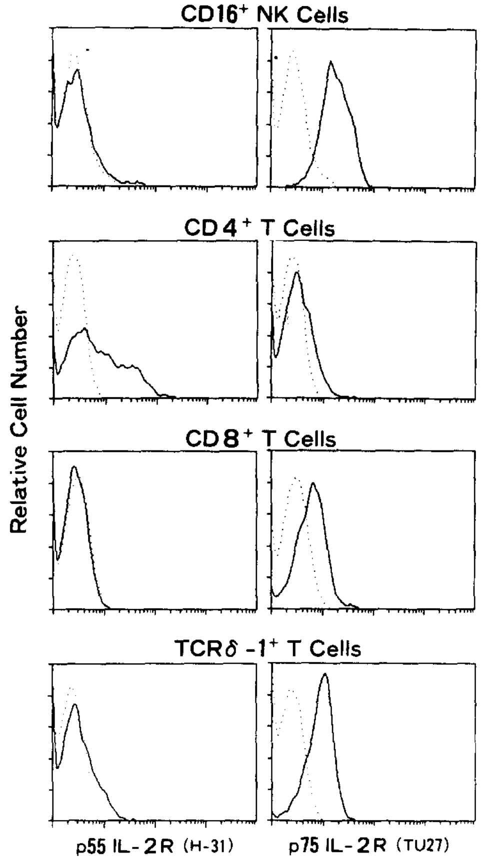

FIGURE 3. Expression of the p55 and p75 IL-2R on T cell subpopulations. PBMC were stained with combinations of biotin-conjugated H-31 or TU27 mAbs and FITC-conjugated anti-CD16 (Leu 11a), -CD4(Leu 3a), -CD8(Leu 2a), or -TCRδ chain (TCRδ-1) mAbs. Biotin-conjugated mAbs were detected by using PE-conjugated streptavidin. Samples were analyzed on a FACScan. Electronic gates were set on CD16$^+$, CD4$^+$, CD8^{bright}, or TCR-δ-1$^+$ (i.e., FITC-stained) lymphocytes, respectively as indicated, at the time of data acquisition, and histograms were generated with red fluorescence (PE staining) of 5,000 cells (*solid lines*). Dotted lines indicate background staining of similarly gated subpopulations with biotin-conjugated control IgG1 mAb.

served with Tγδ cells from five donors, although Tγδ 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$^+$ Tαβ and Tγδ 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 TCRδ-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 $TCR\delta\text{-}1^+$ T cells is predominantly responsible for the direct activation of these cells by IL-2.

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