Tumor Rejection by \textit{in Vivo} Administration of Anti-CD25 (Interleukin-2 Receptor \(\alpha\)) Monoclonal Antibody\(^1\)

Shozaburo Onizuka, Isa Tawara, Jun Shimizu, Shimon Sakaguchi, Teizo Fujita, and Eiichi Nakayama\(^2\)

Department of Parasitology and Immunology, Okayama University Medical School, Okayama 700-8558 [S. O.]; Department of Oncology, Nagasaki University School of Medicine, Nagasaki 852-8523 [S. O.]; Department of Immunopathology, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173-0015 [J. S., S. S.]; and Department of Biochemistry, Fukushima Medical College, Fukushima 960-1295 [T. F.], Japan

\textbf{ABSTRACT}

Immune regulation has been shown to be involved in the progressive growth of some murine tumors. In this study, we demonstrated that a single \textit{in vivo} administration of an amount less than 0.125 mg of anti-CD25 interleukin 2 receptor \(\alpha\) monoclonal antibody (mAb; PC61) caused the regression of tumors that grew progressively in syngeneic mice. The tumors used were five leukemias, a myeloma, and two sarcomas derived from four different inbred mouse strains. Anti-CD25 mAb (PC61) showed an effect in six of the eight tumors. Administration of anti-CD25 mAb (PC61) caused a reduction in the number of CD4\(^+\)CD25\(^+\) cells in the peripheral lymphoid tissues. The findings suggested that CD4\(^+\)CD25\(^+\) immunoregulatory cells were involved in the growth of those tumors. Kinetic analysis showed that the administration of anti-CD25 mAb (PC61), later than day 2 after tumor inoculation caused no tumor regression, irrespective of depletion of CD4\(^+\)CD25\(^+\) immunoregulatory cells. Two leukemias, on which the PC61-treatment had no effect, seemed to be incapable of eliciting effective rejection responses in the recipient mice because of low or no antigenicity.

\textbf{INTRODUCTION}

Immune regulation has been shown to be involved in the progressive growth of some murine tumors. North and Bursuker (1) demonstrated that CD4\(^+\) T cells from BALB/c mice carrying syngeneic methylcholanthrene-induced Meth A sarcoma down-regulated the activation of immune effector cells against the tumor. Similar findings were obtained with other murine tumors (2–4). We previously demonstrated a dominant antigen peptide, pRL1a, that was recognized by cytotoxic T lymphocytes from BALB/c radiation-induced leukemia RL\(\delta\)1 (5). The pRL1a peptide was derived from the 5'-untranslated region of \(c\)-\textit{akt} that became translated by insertion of the long terminal repeat (6). Overexpression of the altered Akt molecules seemed to be responsible for the progressiveness of some murine tumors. North and Bursuker (1) demonstrated that CD4\(^+\)CD25\(^+\) immunoregulatory cells were involved in the progressive growth of tumors. Kinetic analysis showed that the administration of anti-CD25 mAb (PC61), later than day 2 after tumor inoculation caused no tumor regression, irrespective of depletion of CD4\(^+\)CD25\(^+\) immunoregulatory cells. Two leukemias, on which the PC61-treatment had no effect, seemed to be incapable of eliciting effective rejection responses in the recipient mice because of low or no antigenicity.

\textbf{MATERIALS AND METHODS}

\textbf{Mice.} A/St strain of mice was derived from a colony at the Texas University College of Medicine (Houston, TX). BALB/c and B6 mice were purchased from Japan SLC (Shizuoka, Japan). Breeding pairs of AKR/J mice were provided by Dr. T. Shiroishi (National Institute of Genetics, Mishima, Japan). Breeding pairs of CB-17 SCID mice were provided by Dr. K. Kuribayashi (Mie University School of Medicine, Mie, Japan). BALB/c athymic (nu/nu) mice were produced by breeding male athymic (nu/nu) mice with female nu/+ mice at our animal center.

\textbf{Tumors.} The tumor cell lines used and their derivation are listed in Table 1.

\textbf{Antibodies.} Anti-L3T4 (CD4) mAb GK1.5 (15) was provided by Dr. F. Fitch (University of Chicago, Chicago, IL.). Anti-Lyt-2.2 (CD8) mAb 19/178 (16) was provided by Dr. U. Hämmerling (Memorial Sloan-Kettering Cancer Center, New York, NY). Anti-CD25-IL-2Ra mAb produced by hybridoma PC61 (17) was a rat IgG1 antibody. Another anti-CD25 mAb produced by hybridoma, 7D4 (18), was a rat IgM antibody. For \textit{in vivo} administration, anti-CD25 mAb (PC61) was used after purification. The hybridoma ascites produced in CB-17 SCID mice was purified to homogeneity by ammonium sulfate precipitation, followed by chromatography on a DEAE Toyopearl 650S column (Tosoh, Tokyo, Japan). The concentration of IgG was determined from its absorbance at 280 nm as an absorption coefficient value of 1.5.

\textbf{Other reagents.} Anti-L3T4 (CD4) mAb and anti-Lyt-2.2 (CD8) mAb were used in the form of ascites from hybridoma-bearing mice as described previously (19). Depletion of CD4 and/or CD8 T cells by \textit{in vivo} administration of its respective mAb was confirmed as described previously (19). Normal rat IgG was obtained from Caltag (Burlingame, CA).

\textbf{Flow Cytometry.} Cells (1 \(\times\) 10\(^5\)) were washed and incubated with mAb for 30 min at 4°C in 2% FCS-containing PBS. The following mAbs were used: (a) PE-conjugated anti-L3T4 (CD4) mAb (GK1.5; Becton Dickinson Co., Mountain View, CA); (b) PE-conjugated anti-Lyt-2.2 (CD8) mAb (KT15; SeroTek Ltd., Kidlington, Oxford, England); (c) PE-conjugated anti-CD4 mAb (145–2C11); and (d) FITC-conjugated anti-CD25 (IL-2Ra) mAb (7D4; PharMingen Co., San Diego, CA). After treatment, the cells were washed, suspended in PBS, and analyzed on a FACScan (Becton Dickinson).

\textbf{Tumor Assay.} Tumor cells (in 0.2 ml) were injected intradermally into the backs of mice with a 27-gauge needle. Before inoculation of tumor cells, the hair was cut with clippers. The diameter of the tumors was measured with Vernier calipers twice at right angles to calculate the mean diameter.

\textbf{Antibody Administration.} The mice were anesthetized with ether, and a volume of 0.2 ml of mAb diluted in PBS was injected through the retrobulbar venous plexus.

\textbf{RESULTS}

Effect on Tumor Growth of \textit{in Vivo} Administration of Anti-CD25 (IL-2Ra) mAb (PC61). We first investigated the effect of the \textit{in vivo} administration of anti-CD25 mAb (PC61) on a CD25\(^+\)
population in lymphoid tissues. PC61 antibody blocks IL-2 binding to the receptor (17), and anti-CD25 mAb 7D4 does not block IL-2 binding (18). For detection of CD25+ cells in lymph node cells from PC61-treated mice, we used 7D4 with flow cytometry. As shown in Fig. 1 and Fig. 2, CD25+ cells consisted of ~10% CD4+ cells and less than 1% CD8+ cells among the lymph node cells from untreated mice, which was consistent with previous results (11–13). CD4+CD25+ cells reduced maximally on days 3–4 and fully recovered by day 9 after a single in vivo administration of 0.25 mg anti-CD25 mAb (PC61). The reduction was observed in the range of 70–80% at doses between 0.125 and 0.75 mg. For subsequent analyses, we used a single injection of 0.25 mg PC61 on day −4 unless otherwise stated.

The tumors used were listed in Table 1. We used two spontaneously occurring leukemias, two radiation-induced leukemias, a dimethylbenzanthracene-induced leukemia, a mineral oil-induced myeloma and two methylcholanthrene-induced sarcomas. As shown in Fig. 3 and 4, all of the tumors grew progressively in syngeneic mice and eventually killed them. A single administration of 0.25 mg anti-CD25 mAb (PC61) on day −4 caused regression in six of the eight tumors. Administration of normal (control) rat IgG had no effect. No recurrence of tumors was observed thereafter. Among the tumors used, only ASL1 and RL3 were CD25 on the cell surface. Administration of anti-CD25 mAb (PC61) had no effect on the growth of tumors with either CD25+ or CD25− phenotype in BALB/c nu/nu mice.

Next, the timing of the administration of anti-CD25 mAb (PC61) on tumor growth was examined. As shown in Fig. 5, administration on days −4, −2, 0, and 1, but not later than day 2, even with reduction of CD4+CD25+ T cells after MOPC-70A inoculation, caused regression.

The dose effect of anti-CD25 mAb (PC61) on tumor growth was then examined. As shown in Table 2, a single administration of 0.125, 0.25, 0.5, or 0.75 mg on day −4 caused MOPC-70A regression. At doses of 0.03 and 0.06 mg, regression was observed in 0 of 3 and 2 of 3 mice inoculated with the tumor, respectively.

Effect of CD4 and/or CD8 Depletion on Tumor Regression by Anti-CD25 (IL-2Rα) mAb (PC61). To investigate the involvement of CD4 and CD8 T cells in tumor regression caused by the administration of anti-CD25 mAb (PC61), the effect of the coadministration of anti-CD25 mAb (PC61) and anti-CD4 mAb and/or anti-CD8 mAb was investigated. We showed previously (20, 21) that in high responder (BALB/c × B6)F1 mice, CD8 T cells were required for rejection in all of the seven tumors from the BALB/c, B6, and (BALB/c × B6)F1 mice investigated, but the requirement of CD4 T cells differed depending on the tumor. Meth A and MOPC-70A were representative tumors that did and did not require CD4 T cells, respectively. As shown in Fig. 6, in the case of...
MOPC-70A, coadministration with anti-Lyt-2.2 (CD8) mAb, but not anti-L3T4 (CD4) mAb, inhibited the regression by anti-CD25 mAb (PC61). On the other hand, in the case of Meth A, coadministration with either anti-Lyt-2.2 (CD8) mAb or anti-L3T4 (CD4) mAb inhibited the regression.

**Secondary Response in Mice That Rejected Tumors by Anti-CD25 (IL-2Rα) mAb (PC61).** As shown in Fig. 7, no MOPC-70A or RLδ1 growth was observed even at higher doses in BALB/c mice that rejected MOPC-70A or RLδ1, respectively, by anti-CD25 mAb (PC61).

**DISCUSSION**

In this study, we demonstrated that in vivo administration of anti-CD25 mAb (PC61) caused regression of tumors that grew progressively in syngeneic mice and killed them eventually. The tumors used were five leukemias, a myeloma, and two sarcomas derived from four different inbred mouse strains. The effect of anti-CD25 mAb (PC61) was observed on six of the eight tumors. Administration of anti-CD25 mAb (PC61) resulted in a reduction in CD4+CD25+ cells in the peripheral lymphoid tissues. These findings suggested that CD4+CD25+ immunoregulatory cells were involved in the growth of those tumors. Tumor regression was observed even 2–3 months after PC61-treatment. Kinetic analysis showed that the administration of anti-CD25 mAb (PC61) later than day 2 after tumor inoculation caused no tumor regression. This could be due to an insufficient activation of effector cells for proliferating tumor cells by late depletion of CD4+CD25+ T cells. Alternatively, it could be due to a failure in the activation of effector cells by depletion of CD4+CD25+ immunoregulatory cells after tumor antigen stimulation.

We previously identified a dominant rejection antigen peptide recognized by CTL on RLδ1 leukemia cells (5). Irrespective of the presence of the rejection antigen, RLδ1 continued to grow in syngeneic BALB/c mice and killed them eventually. Depletion of CD4+ T cells from the mice resulted in tumor regression (7), consistent with the present findings.

Thymectomy at day 3 after birth caused various autoimmune diseases (9, 22–24). CD4+CD25+ T cells were shown to be responsible for causing the diseases (8, 9). Transfer of CD4+CD25+ cells to those mice inhibited the occurrence of the autoimmune diseases (9). The CD4+CD25+ cells that appeared to represent a distinct lineage (11–13) down-regulated the induction and/or activation of those autoreactive CD4+ T cells from the CD4+CD25− cell pool. Thymectomy at day 3 resulted in the disappearance of CD4+CD25+ cells, which constituted ~10% of the CD4+ T cells in the peripheral lymphoid tissues, which suggests that those cells migrated from the thymus to those tissues on about day 3 after birth (9).

Taguchi and Takahashi (14) demonstrated the depletion of CD25+ cells and the occurrence of autoimmune diseases in (B6 × A/J)F1 mice by in vivo administration of anti-CD25 mAb (PC61) 11 consecutive times every other day at a dose of 2 mg. In our study, a single injection at a dose of 0.125 mg was sufficient to cause regression of the tumors, and no histological indication of autoimmune disease and no autoantibody formation were observed in the mice 3 months after the antibody treatment (data not shown). These findings suggested that the effect of the PC61-treatment seemed to differ between the multitargeted autoimmune responses and the responses against the tumor.

Although the exact mechanisms of suppression by CD4+CD25+ cells in vitro are presently unknown, the in vitro studies by Thornton and Shevach (12) and Takahashi et al. (13) demonstrated that CD4+CD25+ cells suppressed the proliferation of CD4+CD25+ cells by specifically inhibiting the production of IL-2. Moreover, the inhibition required the activation of CD4+CD25+ suppressor cells via T-cell receptor for antigen, and mediation by cell contact but not by cytokines.

Coadministration with anti-CD8 mAb inhibited tumor regression by anti-CD25 mAb (PC61) alone, which suggests that CD8 T cells were responsible for those tumor regressions. Coadministration with anti-CD4 mAb had no effect on the regression of MOPC-70A but inhibited the regression of Meth A by PC61 alone. This suggested that the relative involvement of CD4+ T cells depended on the tumor, probably as helper T cells for the generation of CD8
Fig. 4. Effect of in vivo administration of anti-CD25 mAb (PC61) on tumor growth. ASL1 (1 × 10^6), AKSL2 (2 × 10^6), RL58 (2 × 10^6), EL4 (1 × 10^6), and CMS17 (2 × 10^6) cells were inoculated into the backs of the mice indicated, treated with PBS (control) or PC61 on day −4. Meth A (2 × 10^6) cells were inoculated into the backs of BALB/c mice treated with PC61 or normal rat IgG. Each experimental group consisted of five to six mice. *, the death of all of the mice in the experimental group. Values, means ± SD.

Fig. 5. A, effect of timing of in vivo administration with anti-CD25 mAb (PC61) on tumor growth. MOPC-70A (5 × 10^5) cells were inoculated into the backs of BALB/c mice treated with the mAb on day −4 to day 6 after tumor inoculation. Each experimental group consisted of three mice. *, the death of all of the mice in the experimental group. Values represent the mean. B, reduction in CD4+CD25+ cells by PC61-treatment on days 0, 1, 2, and 4 after MOPC-70A inoculation. Assays were done on day 3 after the mAb treatment by FACScan.
effector cells, and was consistent with our previous results (20, 21). The lack of regression of AKSL2, a spontaneous leukemia derived from an AKR mouse and a RL8, a radiation-induced leukemia derived from a BALB/c mouse by PC61-treatment, together with the normal expression of H-2 class I antigens on those tumors (data not shown) suggested low or no antigenicity of those

<table>
<thead>
<tr>
<th>Antibody dose</th>
<th>0.03 mg</th>
<th>0.06 mg</th>
<th>0.125 mg</th>
<th>0.25 mg</th>
<th>0.5 mg</th>
<th>0.75 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice with tumor regression (Mice inocolated with MOPC-70A)</td>
<td>0/3</td>
<td>2/3</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
</tbody>
</table>

**Table 2** Tumor regression by in vivo administration of anti-CD25 (IL-2Ra) mAb (PC61)

---

Fig. 6. Effect of CD4 and/or CD8 depletion on tumor rejection by PC61. MOPC-70A (5 × 10^5) and Meth A (2 × 10^5) cells were inoculated into the backs of BALB/c mice treated with PC61 on day −4, and anti-L3T4 (CD4) mAb and/or anti-Lyt-2.2 (CD8) mAb on day −7 and day −4. Each experimental group consisted of five to six mice. *, the death of all of the mice in the experimental group. Values, means ± SD.

Fig. 7. Secondary tumor rejection response in PC61-treated mice. MOPC-70A (5 × 10^5) and RL8 (2 × 10^5) cells were inoculated into the backs of BALB/c mice treated with PBS (control) and PC61 on day −4. Mice treated with PC61 were challenged with MOPC-70A (1 × 10^5) or RL8 (1 × 10^5) cells 4–6 weeks after primary tumor rejection. O and □, normal (control) BALB/c mice. Each experimental group consisted of five to six mice. *, the death of all of the mice in the experimental group. Values, means ± SD.
tumors for eliciting effective rejection responses in syngeneic mice.

ACKNOWLEDGMENTS

We thank Drs. O. Taguchi and T. Takahashi (Aichi Cancer Institute, Nagoya, Japan) for providing antibodies. We also thank M. Isobe for excellent technical assistance and J. Mizuuchi for preparation of the manuscript.

REFERENCES

Tumor Rejection by *in Vivo* Administration of Anti-CD25 (Interleukin-2 Receptor α) Monoclonal Antibody


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/13/3128

Cited articles
This article cites 26 articles, 17 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/13/3128.full#ref-list-1

Citing articles
This article has been cited by 100 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/13/3128.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.