Tumor Rejection by *in Vivo* Administration of Anti-CD25 (Interleukin-2 Receptor α) Monoclonal Antibody[1]

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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 *in vivo* administration of an amount less than 0.125 mg of anti-CD25 interleukin 2 receptor α 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.

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 on BALB/c radiation-induced leukemia RL♂1 (5). The pRL1a peptide was derived from the 5′-untranslated region of c-*akt* that became translated by insertion of the long terminal repeat (6). Overexpression of the altered Akt molecules seemed to induce $CD4^+$ immunoregulatory cells, which resulted in progressive RL♂1 growth in BALB/c mice. *In vivo* depletion of $CD4^+$ T cells from BALB/c mice caused RL♂1 regression (7).

Recently, $CD4^+CD25^+$ cells have been shown to represent a unique population of immunoregulatory cells (8–13). Transfer of BALB/c spleen cells depleted of $CD25^+$ cells into BALB/c *nu/nu* mice induced various autoimmune diseases (11). In addition, *in vivo* administration of anti-CD25 mAb[3] induced autoimmune diseases in (B6 × A/J)F$_1$ mice (14).

In this study, we investigated the effect of *in vivo* administration of anti-CD25 mAb on the growth of eight tumors—RL♂1 and four other leukemias, a myeloma, and two fibrosarcomas—that grew progressively in syngeneic mice. We found that a single injection of less than 0.125 mg of anti-CD25 mAb (PC61) caused regression in six of the eight tumors, including RL♂1. After antibody treatment, a reduction in the number of $CD4^+CD25^+$ cells was observed by flow cytometry, which suggested that effective tumor rejection responses resulted from a depletion of $CD4^+CD25^+$ immunoregulatory cells.

MATERIALS AND METHODS

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.

Tumors. The tumor cell lines used and their derivation are listed in Table 1.

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-2Rα 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 *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.

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 *in vivo* administration of its respective mAb was confirmed as described previously (19). Normal rat IgG was obtained from Caltag (Burlingame, CA).

Flow Cytometry. Cells (1×10^6) 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; Serotec Ltd., Kidlington, Oxford, England); (*c*) PE-conjugated anti-CD3ε mAb (145–2C11); and (*d*) FITC-conjugated anti-CD25 (IL-2Rα) mAb (7D4; PharMingen Co., San Diego, CA). After treatment, the cells were washed, suspended in PBS, and analyzed on a FACScan (Becton Dickinson).

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.

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.

RESULTS

Effect on Tumor Growth of *in Vivo* Administration of Anti-CD25 (IL-2Rα) mAb (PC61). We first investigated the effect of the *in vivo* administration of anti-CD25 mAb (PC61) on a $CD25^+$

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Table 1 *Tumors used in this study*

Tumor	Strain	Description	Expression of CD25	Reference
ASL1	A	Spontaneous leukemia	+	(25)
AKSL2	AKR	Spontaneous leukemia	−	(26)
RL♂1	BALB/c	Radiation-induced leukemia	+	(27)
RL♀8	BALB/c	Radiation-induced leukemia	−	(27)
EL4	B6	Dimethylbenzanthracene-induced leukemia	−	(28)
MOPC-70A	BALB/c	Mineral oil-induced myeloma	−	(29)
Meth A	BALB/c	Methylcholanthrene-induced fibrosarcoma	−	(30)
CMS17	BALB/c	Methylcholanthrene-induced fibrosarcoma	−	(31)

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 dimethyl-benzanthracene-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 RL♂1 expressed 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)F_1 mice, CD8 T cells were required for rejection in all of the seven tumors from the BALB/c, B6, and (BALB/c × B6)F_1 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
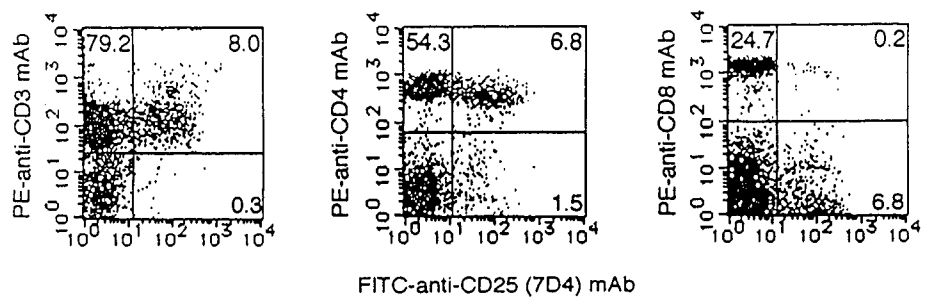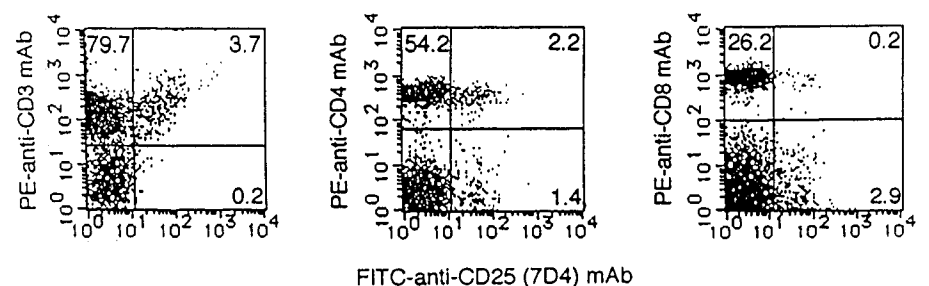
untreated

Fig. 1. Flow cytometry analysis. Lymph node cells from BALB/c mice that were untreated or treated with 0.25 mg PC61 on day −4 were analyzed by FACScan.
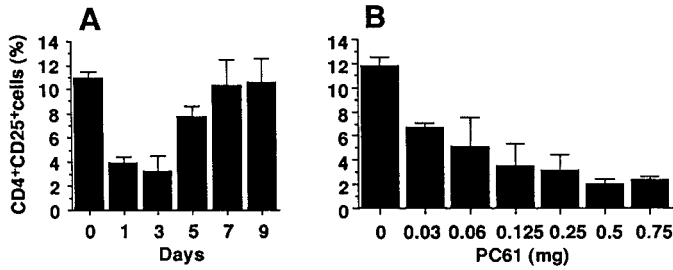
PC61-treated

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Fig. 2. CD4$^+$CD25$^+$ cells (%) 1, 3, 5, 7, and 9 days after the administration of 0.25 mg PC61 (*A*) and those on day 3 after the administration of various doses of PC61 (*B*) in lymph node cells from BALB/c mice. Assays were done by FACScan as shown in Fig. 1. Each experimental group consisted of three mice. *Values*, means ± SD.

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 RL♂1 growth was observed even at higher doses in BALB/c mice that rejected MOPC-70A or RL♂1, 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 RL♂1 leukemia cells (5). Irrespective of the presence of the rejection antigen, RL♂1 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)F$_1$ 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 vivo* 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
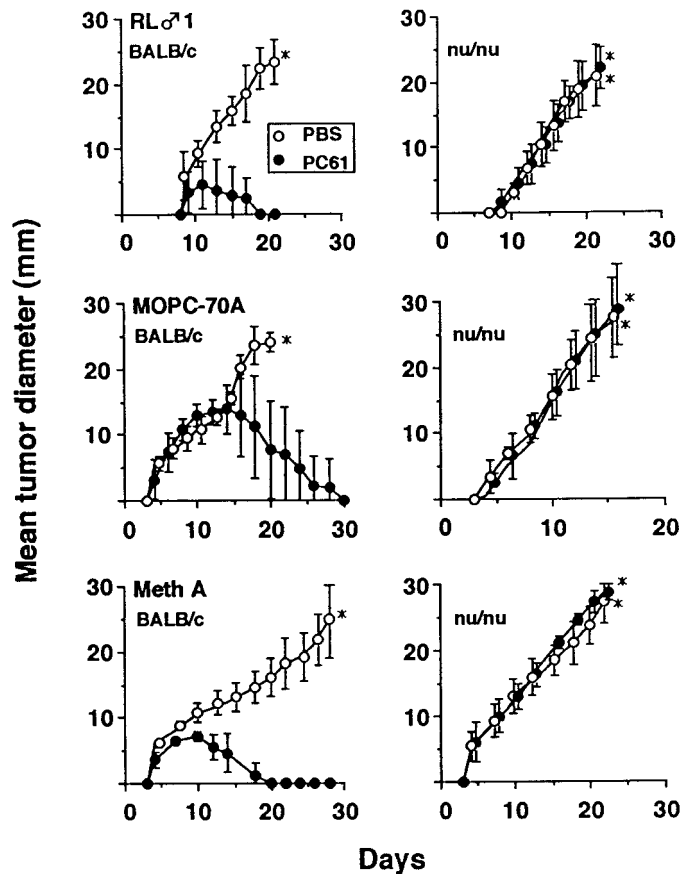

Fig. 3. Effect of *in vivo* administration of anti-CD25 mAb (PC61) on tumor growth. RL♂1 (2 × 10^5), MOPC-70A (5 × 10^5) or Meth A (2 × 10^5) cells were inoculated into the backs of BALB/c or BALB/c nu/nu mice treated with PBS (control) or PC61 on day −4, and the tumor growth was observed. Each experimental group consisted of five to six mice. *, the death of all of the mice in the experimental group. *Values*, means ± SD.
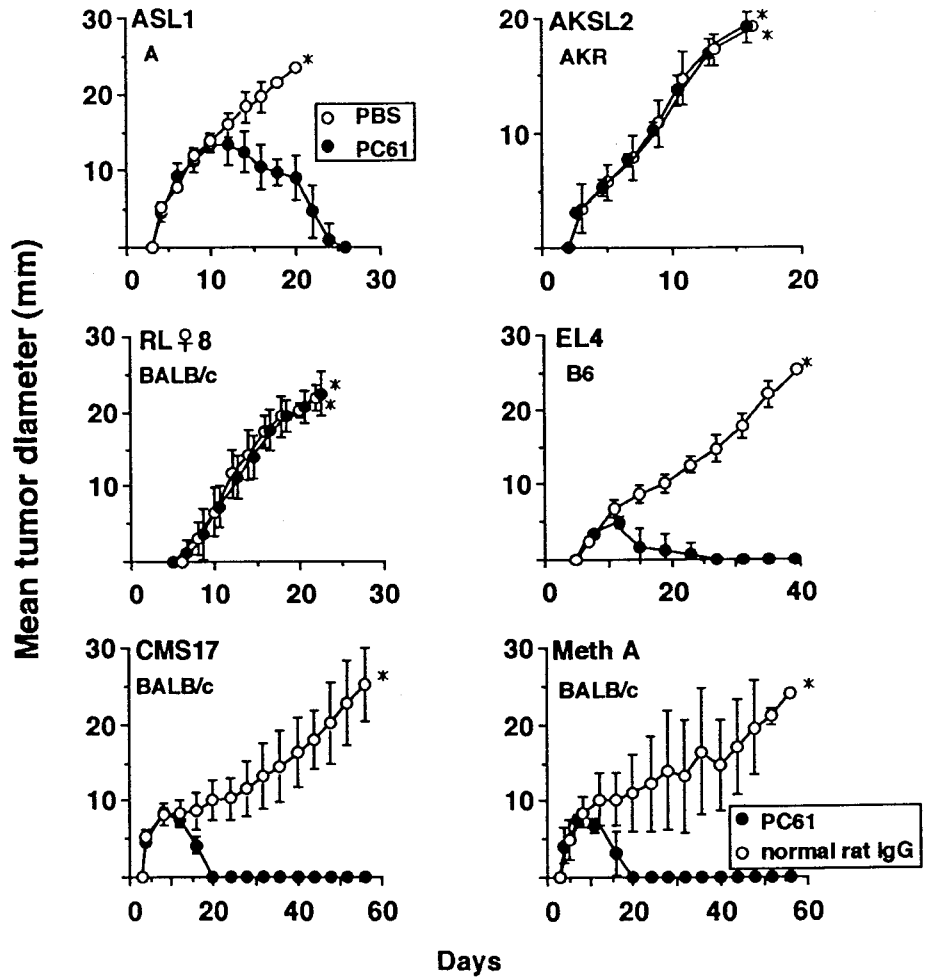
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Fig. 4. Effect of *in vivo* administration of anti-CD25 mAb (PC61) on tumor growth. ASL1 (1×10^6), AKSL2 (2×10^5), RL♀8 (2×10^5), EL4 (1×10^5), and CMS17 (2×10^6) cells were inoculated into the backs of the mice indicated, treated with PBS (control) or PC61 on day -4. Meth A (2×10^5) 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.
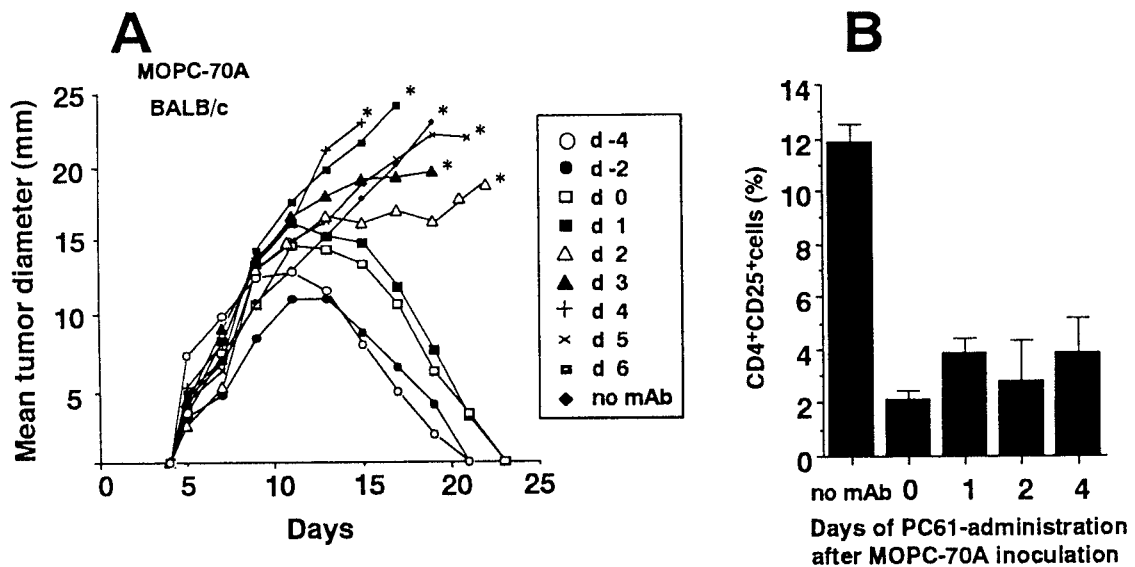

Fig. 5. *A*, effect of timing of *in vivo* administration with anti-CD25 mAb (PC61) on tumor growth. MOPC-70A (5×10^5) cells were inoculated into the backs of BALB/c mice treated with the mAb on day -4 to day 6 after tumor inoculation. Each experimental group consisted of three mice. ∗, the death of 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.

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

Antibody dose	0.03 mg	0.06 mg	0.125 mg	0.25 mg	0.5 mg	0.75 mg
Mice with tumor regression / Mice inoculated with MOPC-70A	0/3	2/3	3/3	3/3	3/3	3/3

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 RL♀8, 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
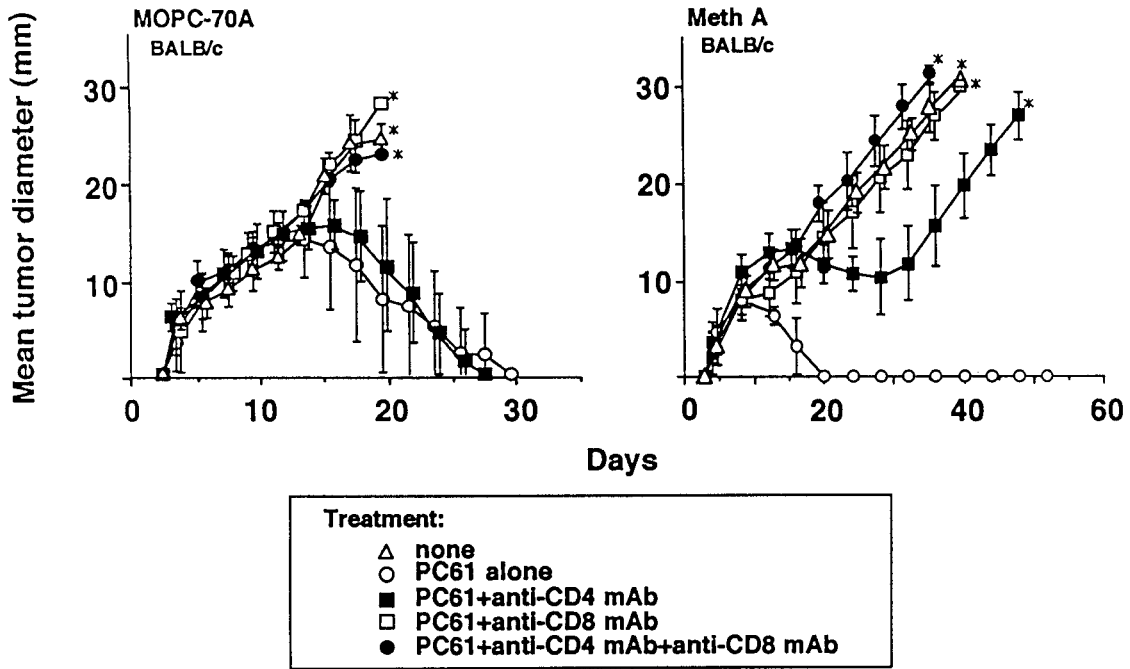

Fig. 6. Effect of CD4 and/or CD8 depletion on tumor rejection by PC61. MOPC-70A (5×10^5) and Meth A (2×10^5) cells were inoculated into the backs of BALB/c mice treated with PC61 on day -4, and anti-L3T4 (CD4) mAb and/or anti-Lyt-2.2 (CD8) mAb on day -7 and day -4. Each experimental group consisted of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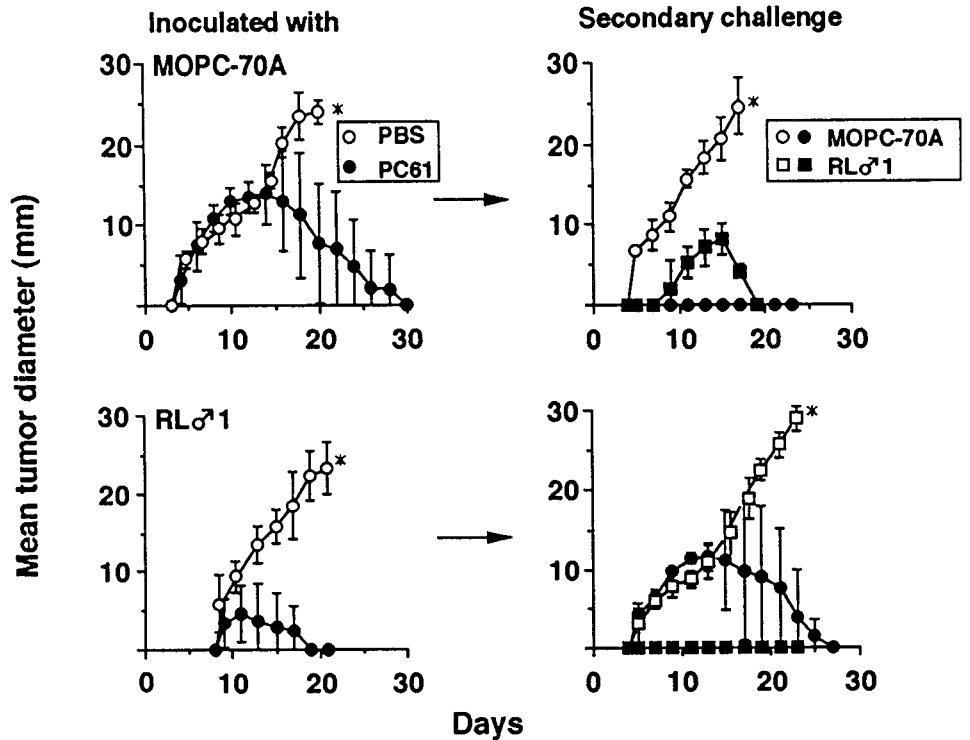

Fig. 7. Secondary tumor rejection response in PC61-treated mice. MOPC-70A (5×10^5) and RL♂1 (2×10^5) cells were inoculated into the backs of BALB/c mice treated with PBS (control) and PC61 on day -4. Mice treated with PC61 were challenged with MOPC-70A (1×10^6) (●) or RL♂1 (1×10^6) (■) cells 4–6 weeks after primary tumor rejection. ○ 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.

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tumors for eliciting effective rejection responses in syngeneic mice.

REFERENCES

1. North, R. J., and Bursuker, I. Generation and decay of the immune response to a progressive fibrosarcoma. I. Ly-1$^+$2$^-$ suppressor T cells down-regulate the generation of Ly-1$^-$2$^+$ effector T cells. J. Exp. Med., *54:* 1295–1311, 1984.
2. Awwad, M., and North, R. J. Immunologically mediated regression of a murine lymphoma after treatment with anti-L3T4 antibody. A consequence of removing L3T4$^+$ suppressor T cells from a host generating predominantly Lyt-2$^+$ T cell-mediated immunity. J. Exp. Med., *168:* 2193–2206, 1988.
3. North, R. J., and Dye, E. S. Ly-1$^+$2$^-$ suppressor T cells down-regulate the generation of Ly-1$^-$2$^+$ effector T cells during progressive growth of the P815 mastocytoma: Immunology, *54:* 47–56, 1985.
4. Rakhmilevich, A. L., North, R. J., and Dye, E. S. Presence of CD4$^+$ T suppressor cells in mice rendered unresponsive to tumor antigens by intravenous injection of irradiated tumor cells. Int. J. Cancer, *55:* 338–343, 1993.
5. Uenaka, A., Ono, T., Akisawa, T., Wada, H., Yasuda, T., and Nakayama, E. Identification of a unique antigen peptide pRL1 on BALB/c RL♂1 leukemia recognized by cytotoxic T lymphocytes and its relation of the *akt* oncogene. J. Exp. Med., *180:* 1599–1607, 1994.
6. Wada, H., Matsuo, M., Uenaka, A., Shimbara, N., Shimizu, K., and Nakayama, E., Rejection antigen peptides on BALB/c RL♂1 leukemia recognized by cytotoxic T lymphocytes: derivation from the normally untranslated 5′ region of the c-*akt* proto-oncogene activated by long terminal repeat. Cancer Res., *55:* 4780–4783, 1995.
7. Matsuo, M., Wada, H., Honda, S., Tawara, I., Uenaka, A., Kanematsu, T., and Nakayama, E. Expression of multiple unique rejection antigens on murine leukemia BALB/c RL♂1 and the role of dominant Akt antigen for tumor escape. J. Immunol., in press, 1999.
8. Sakaguchi, S., Fukuma, K., Kuribayashi, K., and Masuda, T. Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. J. Exp. Med., *161:* 72–82, 1985.
9. Asano, M., Toda, M., Sakaguchi, N., and Sakaguchi, S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. J. Exp. Med., *184:* 387–396, 1996.
10. Suri-Payer, E., Amer, A. Z., Thornton, A. M., and Shevach, E. M. CD4$^+$CD25$^+$ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. J. Immunol., *160:* 1212–1218, 1998.
11. Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J. Immunol., *155:* 1151–1164, 1995.
12. Thornton, A. M., and Shevach, E. M. CD4$^+$CD25$^+$ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. J. Exp. Med., *188:* 287–296, 1998.
13. Takahashi, T., Kuniyasu, Y., Toda, M., Sakaguchi, N., Itoh, M., Iwata, M., Shimizu, J., and Sakaguchi, S. Immunologic self-tolerance maintained by CD25$^+$CD4$^+$ natu-
rally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int. Immunol., *10:* 1969–1980, 1998.
14. Taguchi, O., and Takahashi, T. Administration of anti-interleukin-2 receptor α antibody *in vivo* induces localized autoimmune disease. Eur. J. Immunol., *26:* 1608–1612, 1996.
15. Dialynas, D. P., Quan, Z. S., Wall, K. A., Pierres, A., Quintans, J., Loken, M. R., Pierres, M., and Fitch, F. W. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. J. Immunol., *131:* 2445–2451, 1983.
16. Nakayama, E., Dippold, W., Shiku, H., Oettgen, H. F., and Old, L. J. Alloantigen-induced T-cell proliferation: Lyt phenotype of responding cells and blocking of proliferation by Lyt antisera. Proc. Natl. Acad. Sci. USA, *77:* 2890–2894, 1980.
17. Lowenthal, J. W., Corthesy, P., Tougne, C., Lees, R., MacDonald, H. R., and Nabholz, M. High and low affinity IL-2 receptors: analysis by IL-2 dissociation rate and reactivity with monoclonal anti-receptor antibody PC61. J. Immunol., *135:* 3988–3994, 1985.
18. Ortega, G., Robb, R. J., Shevach, E. M., and Malek, T. R. The murine IL-2 receptor. I. Monoclonal antibodies that define distinct functional epitopes on activated T cells and react with activated B cells. J. Immunol., *133:* 1970–1975, 1984.
19. Yoshimura, A., Shiku, H., and Nakayama, E. Rejection of an IA$^+$ variant line of FBL-3 leukemia by cytotoxic T lymphocytes with CD4$^+$ and CD4$^-$CD8$^-$ T cell receptor-αβ phenotypes generated in CD8-depleted C57BL/6 mice. J. Immunol., *150:* 4900–4910, 1993.
20. Udono, H., Mieno, M., Shiku, H., and Nakayama, E. The roles of CD8$^+$ and CD4$^+$ cells in tumor rejection. Jpn. J. Cancer Res., *80:* 649–654, 1989.
21. Harutsumi, M., Aji, T., Manki, A., Hikida, M., Omori, H., Watanabe, Y., Teramura, Y., Kuribayashi, K., Seino, Y., and Nakayama, E. Rejection of parental Meth A tumor on concomitant inoculation of Meth A cells infected retrovirally with the interferon-γ gene into (BALB/c × C57BL/6)F$_1$ mice. Int. J. Oncol., *7:* 233–238, 1995.
22. Sakaguchi, S., Takahashi, T., and Nishizuka, Y. Study on cellular events in post-thymectomy autoimmune oophoritis in mice. II. Requirement of Lyt-1 cells in normal female mice for the prevention of oophoritis. J. Exp. Med., *156:* 1577–1586, 1982.
23. Moroda, T., Iiai, T., Kawachi, Y., Kawamura, Y. T., Hatakeyama, K., and Abo, T. Restricted appearance of self-reactive clones into T cell receptor intermediate cells in neonatally thymectomized mice with autoimmune disease. Eur. J. Immunol., *26:* 3084–3091, 1996.
24. Wang, J., Griggs, N. D., Tung, K. S., and Klein, J. R. Dynamic regulation of gastric autoimmunity by thyroid hormone. Int. Immunol., *10:* 231–236, 1998.
25. Old, L. J., Boyse, E. A., and Stockert, E. Antigenic properties of experimental leukemias. I. Serological studies *in vitro* with spontaneous and radiation-induced leukemias. J. Natl. Cancer Inst., *31:* 977–986, 1963.
26. Stockert, E., DeLeo, A. B., O'Donnell, P. V., Obata, Y., and Old, L. J. A new cell surface antigen of the mouse related to the dualtropic mink cell focus-inducing class of murine leukemia virus detected by naturally occurring antibody. J. Exp. Med., *149:* 200–215, 1979.
27. Nakayama, E., Shiku, H., Takahashi, T., Oettgen, H. F., and Old, L. J. Definition of a unique cell surface antigen of mouse leukemia RL♂1 by cell-mediated cytotoxicity. Proc. Natl. Acad. Sci. USA, *76:* 3486–3490, 1979.
28. Gorer, P. A. Studies in antibody response of mice to tumor inoculation. Br. J. Cancer, *4:* 372–379, 1950.
29. Potter, M. The plasma cell tumors and myeloma protein of mice. *In:* H. Busch (ed.), Methods in Cancer Research, pp. 106–157. New York: Academic Press, Inc., 1967.
30. Old, L. J., Boyse, E. A., Clarke, D. A., and Carswell, E. A. Antigenic properties of chemically induced tumors. Ann. NY Acad. Sci., *101:* 80–106, 1962.
31. DeLeo, A. B., Shiku, H., Takahashi, T., John, M., and Old, L. J. Cell surface antigens of chemically induced sarcomas of the mouse. I. Murine leukemia virus-related antigens and alloantigens on cultured fibroblasts and sarcoma cells: description of a unique antigen on BALB/c Meth A sarcoma. J. Exp. Med., *146:* 720–734, 1977.

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Shozaburo Onizuka, Isao Tawara, Jun Shimizu, et al.

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