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Effect of in Vivo Administration of Anti-Lyt-2 mAb and Anti-L3T4 mAb on Tumor Rejection

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INTRODUCTION

Effector cells mediating tumor rejection were controversial. Several investigators have shown that by utilizing adoptive transfer into adult, thymectomized, bone marrow reconstituted (ATXBM) or athymic mice, purified Lyt-2+ cells or cloned Lyt-2+ cytotoxic T-cells were capable for tumor rejection (1-6). On the other hand, Lyt-2- (L3T4+) cells have also been shown to mediate tumor rejection (7,8). These studies suggested that either Lyt-2+ or L3T4+ cells appeared to be capable of mediating the rejection. However, it is still unknown whether either type of T-cells is predominantly involved or either Lyt-2+ or L3T4+ cells themselves are fully capable of mediating the rejection. In this study, by eliminating Lyt-2+ cells and/or L3T4+ cells in vivo by administrating anti-Lyt-2.2 mAb, anti-L3T4 mAb or both, we directly investigated the T-cell type that was involved in the effector mechanisms of tumor rejections.

MATERIALS AND METHODS

Mice. C57BL/6 (B6), BALB/c, and (BALB/c x C57BL/6) F₁ (CB6F₁) mice were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). B6-Lyt-1.1 and B6-Lyt-2.1, 3.1 mice were originally provided by Dr. E.A. Boyse, Memorial Sloan-Kettering Cancer Cancer, New York and bred in our laboratory. The Lyt phenotypes of B6, B6 Lyt congenic, and BALB/c mice are as follows: B6, Lyt-1.2,2.2,3.2; B6-Lyt-1.1, Lyt-1.1,2.2,3.2; B6-Lyt-2.1,3.1, Lyt-1.2,2.1,3.1; BALB/c, Lyt-1.2,2.2,3.2.

Tumors. B6RV2 and BALBRVD leukemias induced by injection of neonatal B6 and BALB/c mice respectively, with radiation leukemia virus (9). RLc71 is a radiation-induced leukemia of BALB/c origin (10,11). These tumors were maintained in ascites form in the strain of origin.

Monoclonal Antibodies. Anti-L3T4 mAb, a rat antibody of the IgG 2b immunoglobulin class, produced by hybridoma GK1.5 (12,13), was kindly provided by Dr. F. Fitch, University of Chicago (Chicago, IL). Anti-Lyt-2.2 mAb and other mAb used have been described (14). The titers of both anti-L3T4 and anti-Lyt-2.2 mAb determined by antibody-mediated complement-dependent cytotoxicity assay were 1 : 20,000. These antibodies were used in the form of ascites from hybridoma bearing mice. The concentrations of anti-L3T4 and anti-Lyt-2.2 mAb in pooled ascites were 2.8 and 7.1 mg/ml, respectively, as quantified by protein assay (Bio-Rad Laboratories, Richmond, CA), and by quantitative cellulose acetate electrophoresis.

Tumor Assay. Tumors were harvested in Eagles' minimum essential medium (MEM) and washed twice with medium. Then the desired number of tumor cells (in 0.2 ml) was injected intradermally into the backs of mice through a 30-gauge stainless steel needle. Before inoculation of tumor cells, the hair was shaved with
clippers. The diameters of tumors were measured with vernier calipers, twice, at right angles, to calculate the mean diameter.

**Intravenous Injection of Antibody.** Mice were anesthetized with ether and injected through the retrobulbar venous plexus with 0.2 ml of antibodies (ascites), diluted 1:4 with MEM, through a 1-ml disposable tuberculin syringe fitted with a 26-gauge needle (Terumo, Inc., Tokyo).

**Antibody-mediated, Complement-Dependent Cytotoxicity.** Tests were performed as described previously (15). Before tests, dead cells were removed by density gradient centrifugation in bovine serum albumin (16).

**RESULTS**

Clearance of anti-Lyt-2.2 mAb and anti-L3T4 mAb after in vivo administration. Clearance of anti-Lyt-2.2 and anti-L3T4 mAb in mice was studied by the cytotoxic test with B6 thymocytes as target cells, using serum specimens obtained every other day after in vivo administration of mAb. As shown in Fig. 1, anti-Lyt-2.2 mAb remained until day 30 and disappeared around day 33-35. On the other hand, anti-L3T4 mAb disappeared around day 10. Because of rather rapid clearance of L3T4 mAb, we injected L3T4 mAb on days 0, 4 and 14, whereas Lyt-2.2 mAb on days 0 and 4 in subsequent study.

Direct phenotyping of lymph node cells after in vivo administration of anti-Lyt-2.2 mAb and anti-L3T4 mAb. Change of Lyt-2+ cells and L3T4+ cells in lymph node after in vivo administration of anti-Lyt-2.2 mAb or anti-L3T4 mAb was then investigated (Fig. 2). Lyt-2+ cells

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**Fig. 1.** Clearance of anti-Lyt-2.2 mAb (A) and anti-L3T4 mAb (B) after in vivo administration was examined by antibody-mediated, complement-dependent cytotoxicity assays. Target cells were B6 thymocytes.

**Fig. 2.** Phenotypic change of T-cells in lymph node after in vivo administration of anti-Lyt-2.2 mAb (A) and anti-L3T4 mAb (B) was examined by antibody-mediated, complement-dependent cytotoxicity assays. ●, anti-Thy-1.2 mAb; ■, anti-L3T4 mAb; ▲, anti-Lyt-2.2 mAb; ○, background lysis. N, the reaction of lymph node cells from untreated mice.
Effect of in vivo administration of anti-Lyt-2.2 mAb or anti-L3T4 mAb on tumor rejection. The rejections of a radiation induced leukemia BALBRLcν1 and a RadLV-induced leukemia BALBRVD by CB6F1 recipient mice were studied (Fig. 4). The rejection was blocked by in vivo administration of anti-Lyt-2.2 mAb. Addition of anti-L3T4 mAb to anti-Lyt-2.2 mAb did not alter the results. The rejections were not affect-
ed by the administration of anti-L3T4 mAb alone. These results indicated that Lyt-2+ cells were fully capable of mediating rejection of these tumors and L3T4+ cells appeared not to be involved in rejection. We observed blocking of tumor rejection in mice given only a single injection of Lyt-2 mAb on day 0. Therefore, we investigated the effect of antibody injection at different stages during the course of tumor rejection. As shown in Fig. 5, a single injection of Lyt-2.2 mAb on day 9 after inoculation of B6RV2 was effective for blocking rejection.

**Fig. 5.** Effect of Lyt-2 mAb injected at different stages during the course of tumor regression. CB6F1 mice were given a single injection of Lyt-2.2 mAb on day 0 (A), or 9 (B) after transplantation of 1 x 10^6 B6RV2 cells. (C) Untreated control.

**DISCUSSION**

In the present study, we demonstrated that the rejection of BALBRVD, BALBRL31 and B6RV2 tumors in semisyngeneic recipients was blocked by anti-Lyt-2 mAb, but not anti-L3T4 mAb administered in vivo without exogenous complement. It has been widely accepted that cytotoxic T cells are effector cells in tumor rejection. Recently, however, some investigators have raised questions about the functional role of Lyt-2+ cells as effectors in rejection of allografts (17) or syngeneic tumors (18), by demonstrating that immunity is passively transferred to immunodeficient mice by spleen cells from which Lyt-2+ cells had been eliminated by treatment with anti-Lyt-2 mAb and complement. However, it was also shown that the adult thymectomized, irradiated, and bone marrow-reconstituted mice used in these studies were not truly T cell deficient (19) and that, in fact, host-derived cytotoxic T cells immune to the allograft were recovered from mice to which Lyt-2+-depleted populations were adoptively transferred (20). Thus, it was possible that Lyt-2+ cells are involved in graft rejection. Recently (21), it was reported that immunity to syngeneic fibrosarcoma can be adoptively transferred by Lyt-2+ cells. Consistent with these findings, immunity is also adoptively transferred by infusion of interleukin-2-dependent cytotoxic T cells to syngeneic tumors (22, 23) and allogeneic tumors (24) or tissue (25). Although our results support these findings and demonstrate directly that Lyt-2+ cells are essential for tumor rejection by syngeneic and semisyngeneic recipients, it is still unknown by which mechanism Lyt-2 mAb cause blocking, or at which step of the rejection process Lyt-2 mAb are operative. The finding that a single injection of Lyt-2 mAb on day 0-9 after tumor transplantation effectively blocked rejection suggests that effector cells were functionally blocked rather than that the generation of these cells was inhibited. Previously, we and others demonstrated that in vitro T cell cytotoxicity (26-30) and proliferation (31, 32) in response to alloantigen stimulation, were blocked by anti-Lyt-2 mAb without added complement. We suggested that molecules bearing Lyt-2 and-3 determinants may be involved in T cell recognition. The finding of Lyt-2 blocking of tumor rejection is consistent with these in vitro effects of anti-Lyt-2.

The alteration of the T cell population in the lymph node and spleen of mice injected with mAb is also consistent with findings that, in vitro cultures of H-2 antigen-stimulated T cells, Lyt-1+2+3+ cells predominate under normal circumstances and Lyt-1+2+3− cells predominate when Lyt-1+2+3+ cells are blocked by Lyt-2 or-3 antiserum in the absence of complement (31). The decrease of Lyt-2+ cells and L3T4+ cells in mice injected with Lyt-2 and L3T4 mAb, respectively, did not appear to be due to killing of T cells by mAb and complement from the recipient mice, because Thy-1 and Lyt-1 mAb (both with extremely high titers in cytotoxic tests) did not alter the T cell population.
SUMMARY

Effects of in vivo administration of anti-Lyt-2.2 mAb and anti-L3T4 mAb on the population of T-cells in lymph node, generation of cell-mediated cytotoxicity and tumor rejection were investigated. In vivo administration of anti-Lyt-2.2 mAb selectively eliminated Lyt-2+ cells for more than 50 days and that of anti-L3T4 mAb selectively eliminated L3T4+ cells for more than 30 days. Generation of cytotoxicity was blocked totally by prior administration of anti-Lyt-2.2 mAb and slightly by anti-L3T4 mAb. By investigating the effects of these mAbs on tumor growth, involvement of Lyt-2+ and L3T4+ cells for mediating tumor rejections were studied. Rejections of two radiation leukemia virus (RadLV)-induced leukemias, B6RV2 and BALBRV2, and a radiation induced leukemia BALBRC31 by CB6F1 mice were blocked totally by anti-Lyt-2 mAb but not anti-L3T4 mAb. These results indicated that Lyt-2+ cells were themselves fully capable of mediating rejection of these tumors. On the other hand, L3T4+ cells were not capable of mediating rejection. Injection of anti-Lyt-2.2 mAb on day 9 after tumor inoculation was effective for blocking rejection, suggesting that the effector cells were functionally blocked rather than the generation of those cells was inhibited.

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