Brief Definitive Report

CELL SURFACE ANTIGENS OF MURINE LEUKEMIAS INDUCED BY RADIATION LEUKEMIA VIRUS

Recognition of Individually Distinct Cell Surface Antigens by Cytotoxic T Cells on Leukemias Expressing Crossreactive Transplantation Antigens

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Antigens specific for individual tumors were first discovered in experiments that showed the rejection of chemically induced sarcomas in mice and rats that had been immunized with the same tumor (1). Subsequently, individually distinct antigens were also found in transplantation studies of murine reticulum cell sarcomas and mammary tumors. Detailed study of these unique antigens has long been hampered by the considerable difficulties encountered in attempts to detect them in vitro. Success was first reported in a study of the methylcholanthrene-induced BALB/c sarcoma Meth A. Hyperimmunization of syngeneic mice with this tumor resulted in the production of antibodies recognizing a cell surface antigen whose expression showed the same restriction as the antigen detected in transplantation experiments (2). More recent serological studies of feline leukemias, and of murine sarcomas induced by the Rous sarcoma virus, have also defined individually distinct cell surface antigens expressed only on the tumor used for immunization. With the advances in the serological analysis of human tumors brought about by the development of autologous typing, unique cell surface antigens have also been found on human cancers—melanoma, astrocytoma, and renal cancer (2).

Although individually distinct tumor antigens were initially shown in transplantation experiments generally considered to reflect cellular immunity, there have only been a few reports describing recognition of unique tumor antigens by cytotoxic T cells in vitro (3, 4). We report here that leukemias induced in mice by the radiation leukemia virus (RadLV) express individually distinct antigens recognized by cytotoxic T cells, in addition to crossreacting tumor-specific antigens detected in transplantation experiments.

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Materials and Methods

Mice. The following mouse strains were used: BALB/c, C57BL/6 (B6), and their F_1 hybrids (Memorial Sloan-Kettering Cancer Center, New York); BALB/c Cr Slc, B6 Cr Slc, and their F_1 hybrids (Agricultural Cooperative Association, Shizuoka, Japan); BALB/c NCr, B6 NCr (Charles River Breeding Laboratories Inc., Wilmington, MA).

RadLV Leukemias. B6 mice and BALB/c mice, 1–4 d old, were injected i.p. with RadLV (5, 6). Leukemias developed in 83% of the B6 mice and in 64% of the BALB/c mice. They were maintained in serial transplantation in syngeneic mice, and in stationary suspension culture in RPMI-1640 medium supplemented with 10% FCS.

Antisera. mAbs specific for Thy-1.2, Lyt-1.2, Lyt-2.2, and Lyt-3.2 antigens were used; these antibodies have been described (7).

In Vitro Sensitization of Spleen Cells. As described previously (4). At the end of the incubation period the cells were harvested and used as effector cells in ^{51}Cr release assays for cell-mediated cytotoxicity (^{51}Cr-CMC assays).

Assays and Cell Treatments. ^{51}Cr-CMC assays and competitive inhibition assays, as well as elimination of effector cell subpopulations by antiserum and complement were done as previously described (4).

Results

Resistance to Transplants of RadLV Leukemias in Semisyngeneic F_1 Hybrid Mice. 12 RadLV-induced leukemias were examined, 3 of B6 origin and 9 of BALB/c origin. (BALB/c × C57BL/6)F_1 ($CB6F_1$) mice were injected subcutaneously with 10^4 leukemia cells, and mice that rejected this initial inoculum were subsequently challenged with increasing numbers of leukemia cells. Rejection of the initial leukemia cell inoculum was observed with leukemias B6RV2, B6RV4, BALBRV1, BALBRVA, BALBRVB, and BALBRVD, but not with leukemias B6RV1, BALBRV2, BALBRV3, BALBRV4, BALBRVC, and BALBRVE. Leukemias BALBRVB and BALBRVD were induced in male BALB/c mice, and rejected by female mice but not by male mice, a finding that raises the possibility that male antigens contributed to immunological recognition. In the case of leukemias BALBRV1 and BALBRVA, which were induced in female mice, this possibility need not be considered.

Resistance to Tumor Grafts After Rejection of RadLV Leukemias. Female $CB6F_1$ mice injected subcutaneously with 5×10^5 BALBRVB or BALBRVD leukemia cells showed initial growth and subsequent regression of the leukemia cell inoculum. Most male $CB6F_1$ mice showed progressive tumor growth. After rejection of BALBRVB or BALBRVD leukemia cells, mice were challenged with RadLV leukemias, radiation-induced leukemias, leukemia LSTRA (induced by the Moloney virus), or the methylcholanthrene-induced sarcoma Meth A, all originating in female BALB/c mice except BALBRVB and BALBRVD, which were induced in male mice. Mice that had rejected leukemias BALBRVB or BALBRVD showed growth inhibition of RadLV leukemias BALBRVB, BALBRVD, BALBRV1, BALBRV2, and BALBRV3, and of the radiation-induced leukemia RL♀8. No inhibition of tumor growth was seen in mice challenged with leukemia RL♀6, leukemia LSTRA, or sarcoma Meth A (Table I).

Specificity of Cytotoxic $CB6F_1$ Effector Cells Generated Against RadLV Leukemias BALBRVB and BALBRVD: Results of Direct Tests and Competitive Inhibition Assays. Spleen cells were obtained from $CB6F_1$ mice 2 wk after complete rejection of leukemia BALBRVB, and sensitized in vitro with BALBRVB leukemia cells. Results of direct tests with such cytotoxic effectors are shown in Table II.

TABLE I

Crossreactivity of Transplantation Immunity Against RadLV Leukemias

Tumor used for challenge	Rejection
BALBRVB	+
BALBRVD	+
BALBRV1	+
BALBRV2	+
BALBRV3	+
RL♀6	−
RL♀8	+
LSTRA	−
Meth A	−

After rejecting BALBRVB or BALBRVD, female $CB6F_1$ mice were challenged with 5×10^5 viable tumor cells.

TABLE II

Cytotoxicity of $CB6F_1$ Effector Cells Generated Against RadLV Leukemias BALBRVB or BALBRVD: Results of Direct Tests

Effector cells	Target cells	Percent specific lysis					
		20*	10	5	2.5	1.2	0.6
$CB6F_1$ anti-BALBRVB‡	BALBRVA	5	1	0	0	0	0
	BALBRVB	50	48	44	40	39	27
	BALBRVC	8	0	0	0	0	0
	B6RV2	1	0	0	0	0	0
$CB6F_1$ anti-BALBRVD‡	BALBRVA	0	0	0	0	0	0
	BALBRVB	15	10	5	6	7	—
	BALBRVC	0	0	0	0	0	—
	BALBRVD	76	74	64	49	46	42

* E/T cell ratio; 2×10^4 ^{51}Cr-labeled target cells were used.

‡ Spleen cells from mice that had rejected leukemia BALBRVB or BALBRVD were sensitized in vitro with BALBRVB or BALBRVD cells, respectively.

Cytotoxicity was demonstrable only in tests on the RadLV cells used for immunization, not in tests on any other target cells. These tests included 10 RadLV leukemias and Con A blasts from 12 mouse strains (Table III).

The specificity of the reaction was further analyzed in competitive inhibition assays. Individual tests are shown in Fig. 1. Lysis of BALBRVB leukemia cells by effector cells against leukemia BALBRVB was inhibited only by BALBRVB leukemia cells, not by other RadLV leukemia cells or normal spleen cells and thymocytes (*A* and *B*). Similar results were obtained in competitive inhibition assays using effector cells generated against leukemia BALBRVD (*C* and *D*). A summary of results obtained with leukemia BALBRVB is shown in Table III. The results of competitive inhibition assays with effector cells against leukemia BALBRVD also confirmed the results of direct tests. In both systems, the only cells that inhibited cytotoxic reactivity were the leukemia cells, BALBRVB or BALBRVD, against which the effector cells had been generated, indicating individually distinct specificity of the antigens recognized in the cytotoxic reaction.

T Cell Characteristics of the Cytotoxic Effector Cells Generated Against Leukemias BALBRVB and BALBRVD. The T cell characteristics of the cytotoxic effector cells were defined in experiments eliminating effector cell subpopulations by

TABLE III

Individually Distinct Specificity of CB6F$_1$ Cytotoxic Effector Cells Reactive with RadLV Leukemia BALBRVB: Nonreactive Target Cells in Direct Tests and Competitive Inhibition Tests

RadLV leukemias	Moloney virus leukemia	Con A blasts
BALBRVA	BALB/c LSTRA	BALB/c
BALBRVC		B6
BALBRVD	Methylcholanthrene sarcomas	C3H/He
BALBRVE	BALB/c Meth A	SJL/J
BALBRV1	CMC-1	RIII/2J
BALBRV2	CMC-11	PL/J
BALBRV3	CMC-12	A.CA/Sn
B6RV1	CMC-13	B10WB/Sn
B6RV2		C3H.NB/Sn
B6RV4	Rous virus sarcomas	DBA/1J
	BALB/c C-SA-1M	A/J
Radiation leukemias	C-SA-9F	SM/J
BALB/c RL♂1		
RL♀6	Myelomas	Spleen cells, thymocytes
RL♀8	BALB/c 4T001	BALB/c, B6
	MPC-11	

Comparable results were obtained in tests with effector cells generated against leukemia BALBRVD.

pretreatment with antibodies against T cell differentiation antigens, with addition of complement. Treatment with Thy-1.2, Lyt-2.2, or Lyt-3.2 antibody completely abolished cytotoxic reactivity. Treatment with Lyt-1.2 antibody reduced cytotoxic reactivity but did not eliminate it. A significant decrease of cytotoxicity was also observed when Lyt-2.2 or Lyt-3.2 antibodies were added without complement at the start of the cytotoxicity assay.

Discussion

We have examined the immune response of mice to RadLV-induced leukemias in terms of transplantation immunity in vivo and T cell cytotoxicity in vitro. It appears that the two reactions detect different antigenic systems. CB6F$_1$ female mice preimmunized with RadLV leukemias BALBRVB or BALBRVD showed growth inhibition of five RadLV leukemias when subsequently challenged, indicating the presence of tumor-specific transplantation antigens shared by leukemias induced by the same virus. Male antigens may play a role in the primary rejection of leukemias BALBRVB and BALBRVD (both induced in male mice) by female recipients. They are unlikely to be responsible, however, for the secondary rejection in preimmunized mice, because RadLV leukemias derived from female mice were also rejected.

In contrast to the crossreactivity between RadLV leukemias seen in transplantation experiments, cytotoxic T cells generated against RadLV leukemias BALBRVB or BALBRVD recognized only the cells of the leukemias used for immunization. Shared antigens were not detected in direct assays or inhibition assays on a large panel of leukemias and other tumors of BALB/c origin. Identical results were obtained with these two leukemias, indicating that expression of individually distinct antigens is not restricted to a rare leukemia. Cytotoxic T cells generated against murine leukemia virus (MuLV)-induced leukemias have been reported to recognize antigens shared by several leukemias induced by the same virus (8–12), predominantly type- or subgroup-specific determinants of

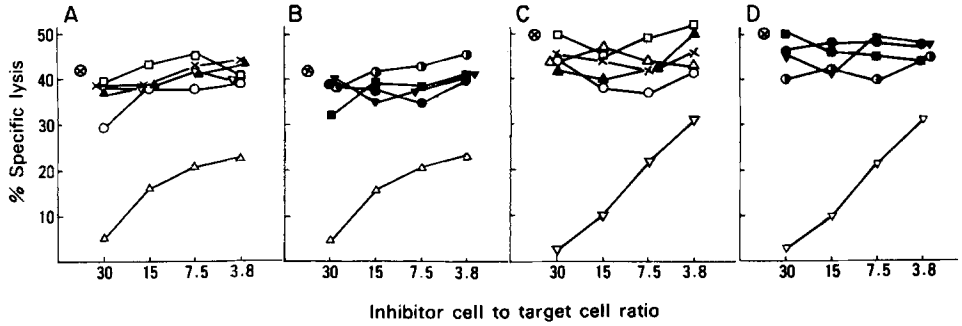

FIGURE 1. Competitive inhibition assays with effector cells generated against leukemias BALBRVB (*A* and *B*) and BALBRVD (*C* and *D*). Unlabeled inhibitor cells were added at indicated ratios to 2×10^4 ^{51}Cr-labeled target cells. E/T ratio was 15:1. Inhibitor cells used: ⊗, none; O, BALBRVA; △, BALBRVB; □, BALBRVC; ▽, BALBRVD; ×, BALBRVE; ▲, B6RV2; ●, BALB/c thymocytes; ■, BALB/c spleen cells; ▼, B6 thymocytes; ◑, B6 spleen cells.

gp70 or other viral structural components (8–11). Compared with the extensive information that exists regarding the specificity of T cell recognition of leukemias induced by Gross and Moloney MuLV, very little is known about RadLV-induced leukemias. However, it is clear from our findings and from other observations (5) that RadLV leukemias express individually distinct cell surface antigens that can be recognized by cytotoxic T cells.

An intriguing aspect of the tumor-host relationship observed in this study is the difference in specificity between T cell cytotoxicity (detecting individually specific antigens) and transplantation immunity (detecting shared antigens). This finding raises questions regarding the role cytotoxic T cells play in the rejection of tumors in vivo. Issues requiring clarification include the phenotypic characteristics of effector cells in the two reactions, the genetic control of induction of effector cell activity, and the operation of genetic restriction in effector cell function. Preliminary results suggest that Lyt-1^+,2^+ and Lyt-1^+,2^- T cells are required for in vivo transfer of crossreactive resistance to RadLV leukemias, and that Lyt-1^+,2^+ and Lyt-1^-,2^+ cells participate in the cytotoxic in vitro reaction that recognizes unique antigens. Establishment of cloned effector cell lines in continuous culture will be useful in the pursuit of these issues.

Summary

The specificity of transplantation immunity and T cell cytotoxicity against leukemias induced by RadLV was examined. Subcutaneous inoculation of two RadLV leukemias induced in BALB/c mice, BALBRVB and BALBRVD, resulted in initial tumor growth in $CB6F_1$ mice, followed by complete tumor regression. Mice that had rejected leukemias BALBRVB or BALBRVD were subsequently challenged with various tumors of BALB/c origin. The growth of all five RadLV leukemias tested, and of one radiation-induced leukemia, was significantly inhibited. Another radiation-induced leukemia, a methylcholanthrene-induced sarcoma, and a leukemia induced by the Moloney leukemia virus,

were not inhibited. The results indicate that RadLV leukemias share cell surface antigens that induce transplantation immunity in vivo. Cytotoxic lymphocytes were generated by coculturing spleen cells from mice that had rejected leukemia BALBRVB or BALBRVD with the corresponding leukemia cells. Direct tests and inhibition tests showed that such cytotoxic cells recognized individually specific antigens on leukemias BALBRVB and BALBRVD, distinct from the shared antigens detected in transplantation experiments. The effector cells in cytotoxicity assays were Thy-1^+, Lyt-$1^{+,-}$, Lyt-2^+, and Lyt-3^+ T cells.

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