Brief Definitive Report

ALLOGENEIC TUMOR REJECTION INDUCED BY THE INTRAVENOUS INJECTION OF Lyt-2$^+$ CYTOLYTIC T LYMPHOCYTE CLONES*

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Allograft and tumor rejection have been shown to be mediated by both humoral (antibody) and cellular mechanisms (1, 2). However, the actual lymphocyte subpopulations involved in the cell-mediated rejection phenomenon remain to be identified. Cytolytic T lymphocytes (CTL) have been considered as a leading candidate because they have been shown to be generated during graft rejection (2), to be highly cytolytic for the relevant target cells in vitro (3), to inhibit tumor growth in vivo when admixed with tumor cells (4), and to accumulate selectively in grafts (5) and tumors (6). Nevertheless, recent reports question the role of CTL in vivo because precursor T cells of the Lyt-2$^-$ phenotype and not Lyt-2$^+$ (cytolytic) cells were shown to mediate murine allograft (7) and rat syngeneic tumor rejection (8). With the possibility of deriving and maintaining in continuous culture clones of functionally active T cells, (9–12), the use of such cloned effector T cells should provide a direct means of resolving these contradictory observations.

Previous experiments testing several allogeneic and syngeneic CTL clones had failed to show a protective effect against tumor grafts after intravenous injection (13, 14), results that might be explained by an inadequate homing pattern (15) and/or limited survival of such clones in vivo (16). In vitro, CTL clones have been found to depend on addition of exogenous T cell growth factor (TCGF) for survival and responsiveness to antigenic stimulation (9–12). In view of recent evidence demonstrating the existence of antigen-driven CTL clones able to proliferate autonomously upon stimulation with alloantigen in the absence of added TCGF (17),[1] several autonomously proliferating CTL clones were selected for the present studies. We demonstrate the ability of certain Lyt-2$^+$ CTL clones, when injected intravenously, to induce allogeneic tumor cell destruction within the peritoneal cavity of immunosuppressed histocompatible mice.

Materials and Methods

Using techniques described in detail elsewhere,[1] CTL clones specific for H-2 K/D alloantigens were isolated from day-5 primary C57BL/6 (H-2^b) anti-P-815 (H-2^d) mixed leukocyte-tumor

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[1] Glasebrook, A. L. 1982. Cytolytic T lymphocyte clones which proliferate autonomously to specific alloantigenic stimulation. I. Frequency, interleukin-2 production and Lyt phenotype. Manuscript submitted for publication.

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cell cultures. Individual blast cells were micromanipulated into microwells containing irradiated P-815 tumor cells, irradiated syngeneic spleen cells, and a source of TCGF.[1] The resultant clones were tested for antigen-induced proliferation in the absence of added TCGF and for cytolytic activity on ^{51}Cr-labeled P-815 tumor cells. Four autonomously proliferating cytolytic clones were selected, expanded in mass culture, and evaluated for the ability to induce tumor cell destruction i.p. in syngeneic, sublethally irradiated (650 rad) mice after intravenous injection (13, 14). Tumor cells were initially labeled with ^{131}I-IUdR (18) and then 2×10^6 cells injected intraperitoneally. Tumor cell survival was monitored by whole-body counting techniques (14, 18) because ^{131}I label released from dead tumor cells is eliminated without reuse. The clones were further studied for specificity of cytolysis using several ^{51}Cr-labeled tumor targets (3), antigen-induced production of lymphokines γ-interferon (IFN-γ) and macrophage-activating factor(s) (MAF) (19), and cell surface phenotype using monoclonal antibodies and flow cytofluorographic analysis (20).

Results

Fig. 1 shows that two of the four clones tested were able to mediate allogeneic tumor cell rejection after intravenous infusion, i.e., clones AL 15 (Fig. 1 A) and AO 37 (Fig. 1 B). It should be noted that the two clones that were active in vivo were derived in independent experiments. Clone AL 15 cells induced a rapid, dose-dependent destruction of labeled tumor cells in the peritoneal cavity. 20 million cloned CTL injected intravenously resulted in the elimination by day 9 of >95% of the ^{131}I-IUdR label initially injected (Fig. 1 A). This dose of cloned T cells resulted in total protection of the mice, as assessed by survival 35 d later, in contrast to 5×10^6 cells that did not yield protection. The control group, which received 20×10^6 normal syngeneic spleen

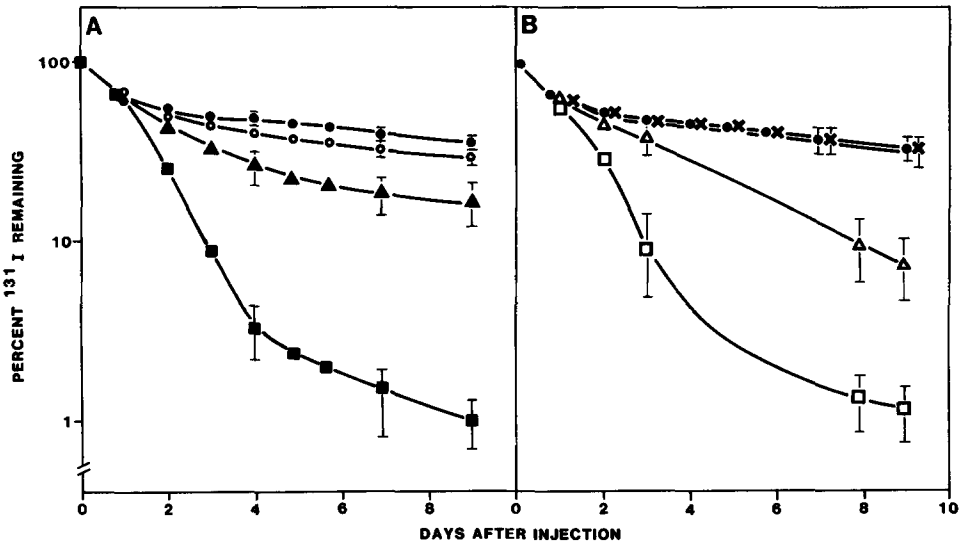

FIG. 1. Allogeneic tumor cell destruction induced by the intravenous injection of Lyt-2$^+$ cytolytic T cell clones directed against H-2 K/D alloantigens. The elimination of ^{131}I-IUdR-labeled (DBA/2) P-815 tumor cells injected intraperitoneally was monitored by whole-body counting techniques (14, 18). Groups of 4–6 sublethally irradiated (650 rad) C57BL/6 mice were injected intravenously (i.v.) with various C57BL/6 (H-2^b) anti-P-815 (H-2^d) CTL clones, followed immediately by 2×10^6 labeled P-815 intraperitoneally. A, (●) 20×10^6 C57BL/6 normal spleen cells i.v.; (○) 1×10^6 clone AL 15 i.v.; (▲) 5×10^6 clone AL 15 i.v.; (■) 20×10^6 clone AL 15 i.v. B, (●) 20×10^6 C57BL/6 normal spleen cells i.v.; (×) 20×10^6 clone AL 33 i.v.; (△) 20×10^6 clone AO 24 i.v.; (□) 20×10^6 clone AO 37 i.v.

cells intravenously, retained 30-fold more radioactivity as measured on day 9, and all mice were dead with ascites by day 18 of experiment. CTL clone AO 37 gave similar results when tested in an independent experiment (Fig. 1 B), whereas clone AO 24 induced only a partial elimination of the labeled P-815 cells. Interestingly, CTL clone AL 33, which was isolated in an identical manner, failed to confer protection after injection of 20×10^6 cells intravenously (Fig. 1 B).

Table I summarizes several in vitro activities exhibited by the CTL clones described in Fig. 1. All clones were highly cytolytic when tested on ^{51}Cr-labeled P-815 (H-2^d) cells, the original stimulating alloantigen, and gave no detectable lysis of syngeneic EL-4 (H-2^b) or of third-party BW5147 (H-2^k) tumor target cells. All clones secreted considerable levels of the lymphokines IFN-γ and MAF during the expansion phase in Costar 24-well plates.

Fig. 2 depicts the cell surface phenotype of AL 15 and AO 37, the two clones that afforded protection, in addition to that of the nonprotective clone AL 33. All CTL clones expressed Lyt-2 but the expression of Lyt-1 was variable, as has been observed previously (11 ,12).

Discussion

Although individual T cell clones specific for SRBC (21), γ, influenza virus (22), or parasite antigens (23) have been shown to function in vivo, this is the first report demonstrating the tumoricidal activity of alloreactive cloned Lyt-2^+ cytolytic T cells in vivo after intravenous injection. Two out of the four autonomously proliferating CTL clones tested induced tumor cell destruction at a site distant from their injection. These results are in contrast to previous attempts in which six CTL clones failed to afford protection (13, 14).

Because the CTL clones analyzed in this study proliferate autonomously in vitro in response to stimulation with alloantigens, in the absence of an added source of TCGF,

TABLE I

Characteristics of Murine CTL Clones Tested for the Ability to Induce Tumor Rejection after Intravenous Injection

	Clone injected	Surface phenotype	Cytolytic activity (LU/10^6 cells)			IFN-γ production	MAF production	Tumor rejection
			P-815 (H-2^d)	EL-4 (H-2^b)	BW5147 (H-2^k)			
						U	*U*	
Experiment 1	AL 15	Thy-1^+, Lyt-1^-2^+	714	<0.3	<0.3	160	69	+++
	AL 33	Thy-1^+, Lyt-1^-2^+	333	<0.3	<0.3	160	61	−
Experiment 2	AO 24	Thy-1^+, Lyt-1^+2^+	625	<0.3	<0.3	640	314	+
	AO 37	Thy-1^+, Lty-1^+2^+	330	<0.3	<0.3	1,280	897	+++

Murine T cell clones were derived by micromanipulation techniques starting from a day 5 C57BL/6 (H-2^b) anti-P-815 (H-2^d) allogeneic mixed leukocyte-tumor culture as described elsewhere.[1] Cytolytic clones that proliferated autonomously when cultured with irradiated P-815 tumor cells plus irradiated T cell-depleted syngeneic spleen cells in the absence of added TCGF were expanded in Costar 24-well plates and tested for ability to induce the rejection of ^{131}I-IUdR-labeled P-815 tumor cells after intravenous injection (14, 18) (Fig. 1), cell surface phenotype (20), (Fig. 2), cytolytic activity on ^{51}Cr-labeled tumor cells expressed as lytic units per 10^6 cloned T cells (3), and production of the lymphokines γ-interferon (IFN-γ) and macrophage-activation factor (MAF) expressed in units per ml relative to a standard preparation (19).

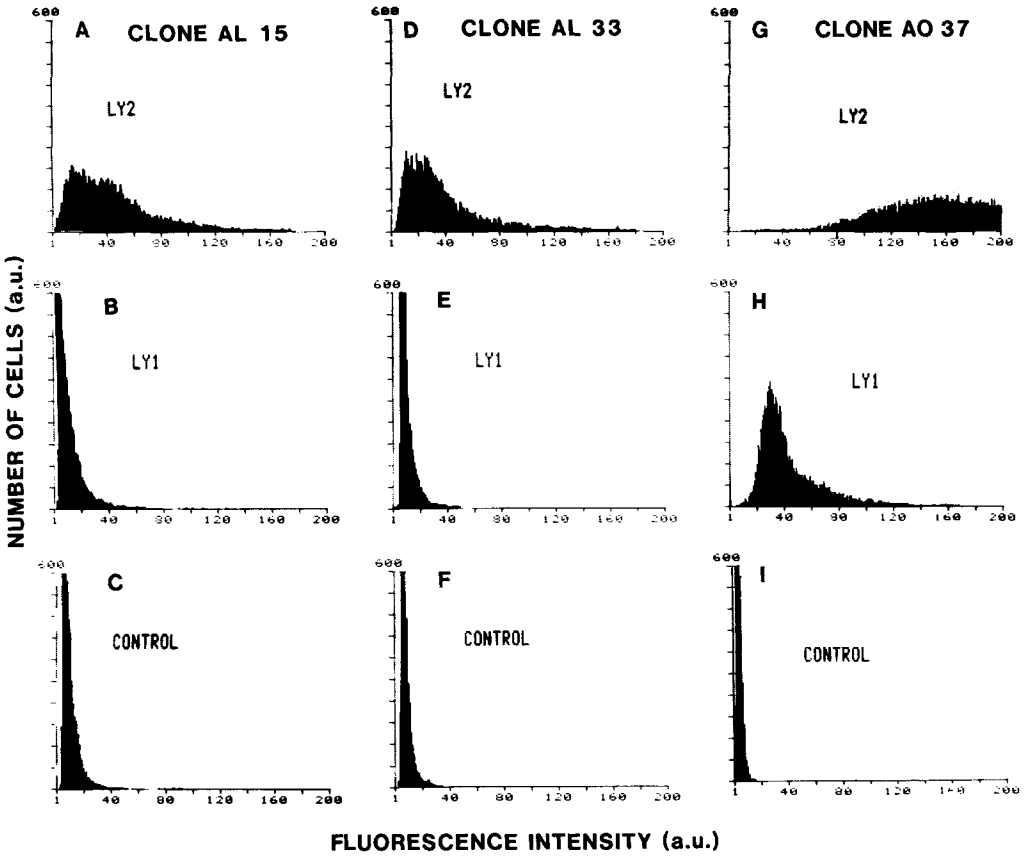

FIG. 2. Cell surface phenotype of CTL clones. 1 million cells of clone AL 15, clone AL 33, or clone AO 37 were incubated with monoclonal rat antibodies against Lyt-2 (A, D, G) or Lyt-1 (B, E, H) followed by FITC-conjugated rabbit anti-rat Ig and analyzed on an FACS II flow cytometer (20). Control samples (C, F, I) were stained with the fluorescent anti-Ig reagent alone. Each histogram represents 10,000 viable cells gated according to forward light scatter. (a.u., arbitrary units).

it is tempting to speculate that this selected subpopulation of Lyt-2^+ CTL effector cells is especially suited to function in vivo after intravenous administration. It should, however, be noted that on a per cell basis, the protective CTL clones were 5–20-fold less efficient at inducing tumor cell rejection in vivo than were equally cytolytic Lyt-2^+ cell populations recovered from day 5 mixed leukocyte cultures (13, 14). Preliminary results using ^{131}I-IUdR-labeled CTL clones demonstrate that up to 70% of the injected cells recovered are trapped in the lungs after intravenous injection (13, unpublished observations). This may be one reason for the lower efficiency of cloned CTL cells vs. the day 5 mixed leukocyte culture effector cells. In addition, these observations may explain the positive results obtained in a previous report, whereby intravenous injection of an influenza-specific CTL clone protected mice from a nasal infection with virus (22).

The mechanism by which the injected effector cells accomplish the actual destruction of the tumor cells in vivo in this model system remains to be elucidated. Although it appears likely to be via direct cytolytic activity, a participation of radioresistant

host cells, e.g., activated macrophages, cannot be ruled out. In addition, all CTL clones tested secreted considerable levels of IFN-γ in vitro. Experiments are in progress to investigate the production of IFN-γ in vivo in this model system. It also remains to be established whether differences in homing patterns between various T cell clones exist, and, if so, what role these differences might play in the functional activities displayed by these clones.

Summary

The in vivo activity of murine Lyt-2^+ cytolytic T lymphocyte clones was assessed in a tumor allograft model system. Mice that had been sublethally irradiated 16 h previously were injected intraperitoneally with ^{131}I-IUdR-labeled tumor cells. Simultaneously, various doses of four cytolytic T cell clones were injected intravenously and the mice monitored for tumor cell elimination by whole-body counting techniques. These four clones had been selected on the basis of their ability to proliferate in response to alloantigens in the absence of added T cell growth factor(s). With two of the four clones tested, rapid elimination of tumor cells within the peritoneal cavity was observed, as early as 48 h after intravenous injection of the cloned T cells.

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