

HYBRID RESISTANCE TO EL-4 LYMPHOMA CELLS

I. Characterization of Natural Killer Cells That Lyse EL-4 Cells and Their Distinction from Marrow-dependent Natural Killer Cells*

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Rejection of hemopoietic transplants in mice has several peculiar immunogenetic features that set it apart from the rejection of solid-tissue allografts (1, 2). The effector system responsible for rejection of marrow transplants is composed of cells that are independent of the thymus for functional differentiation and that are resistant to lethal doses of whole-body irradiation. The antigens relevant in marrow allograft reactions are called hybrid or hemopoietic histocompatibility (*Hh*)¹ antigens. These antigens are not inherited codominantly and are therefore not expressed in heterozygous mice. This pattern of inheritance is responsible for the phenomenon of hybrid resistance, i.e., the rejection of parental bone-marrow cell grafts by irradiated F₁ mice. One of the *Hh* antigens, *Hh-1*, is coded for by genes linked to the D-end of the major histocompatibility complex *H-2* (3). *Hh* incompatibility is necessary, but not sufficient, to induce rejection of hemopoietic grafts. The ability of various inbred mouse strains to reject *Hh*-incompatible marrow transplants is regulated by two or more immune response (*Ir*)-like genes not linked to *H-2* (4).

The anti-*Hh* effector system is still not clearly defined. Because treatment of mice with the long-lived bone-seeking isotope, ⁸⁹Sr, leads to the selective abrogation of marrow allograft reactivity, it has been suggested that the effector cells are marrow-dependent (M) cells (5). Two assay systems mimic some of the peculiar features of marrow graft rejection in vitro. Shearer and Cudkowicz (6) have developed a system in which lymphocytes from F₁ hybrid mice specifically cytotoxic for parental *Hh* antigens can be generated. However, in contrast to in vivo hybrid resistance, the effector cells in this model are T lymphocytes (7). The other in vitro system that shares many features with the in vivo anti-*Hh* effector cells is the natural killer (NK)-cell system. NK cells, present in normal (unimmunized) mice, can lyse many lymphoma cell lines in vitro (8, 9). A detailed comparison of NK-cell activity against YAC-1 lymphoma with marrow allograft reactivity (10) indicated a strong correlation between the two systems. Most impressive is the abrogation of NK activity against

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¹ *Abbreviations used in this paper:* ADCC, antibody-dependent cellular cytotoxicity; CML, cell-mediated lympholysis; complete RPMI-1640 medium, RPMI-1640 with 10% fetal calf serum, penicillin and streptomycin, L-glutamine, sodium pyruvate, and nonessential amino acids; Con A, concanavalin A; CY, cyclophosphamide; E:T, effector to target-cell; *Hh*, hybrid or hemopoietic histocompatibility; *Ir*, immune response; M, marrow-dependent; NK, natural killer; non-M, marrow-independent; PBS, phosphate-buffered saline; pI:pC, polyinosinic:polycytidylic acid; PLL, poly-L-lysine; SE, sheep erythrocytes; SEA, 7S antibody-coated sheep erythrocytes.

YAC-1 cells by treatment of mice with ^{89}Sr , indicating that anti-YAC-1 NK cells are M cells (10, 11). However, unlike marrow allograft reactivity, the lysis of YAC-1 cells in the NK assay did not seem to reflect recognition of *Hh* antigens (10). Recently, a weak NK reactivity against EL-4 (*H-2^b*) lymphoma cells has been described (12). Spleen cells from *H-2D^b* heterozygous F₁ mice lyse EL-4 cells much more efficiently than cells from parental (*H-2^b*) mice. This similarity with in vivo hybrid resistance against *H-2^b* bone-marrow cells suggests that *Hh-1^b* antigens are relevant in the lysis of EL-4 cells in the NK assay. Therefore it appeared to us that the anti-EL-4 NK system may be an excellent candidate for study of anti-*Hh* reactions in vitro.

We report here a detailed characterization of anti-EL-4 NK cells and comparisons between anti-YAC-1 and anti-EL-4 NK cells. Anti-YAC-1 NK cells are M cells but anti-EL-4 NK cells are independent of the marrow microenvironment for functional maturation. Despite the differences in the genetic pattern of lytic interactions and in the effector cells themselves, the data suggest that both EL-4 and YAC-1 cells express *Hh-1^b* antigens. *Hh* antigens may constitute NK recognition structures. In the next paper of this series, we have analyzed the relative roles of the M cells and marrow-independent NK cells in mediating tumor resistance in vivo.²

Materials and Methods

Mice. CBA, C57BL/6 (B₆), DBA/2J, B10.D2n/Sn, B10.A, (C57BL/6 × DBA/2)F1 (B₆D₂F₁), and A/J mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. B10.A (5R) mice were originally obtained from Dr. G. Cudkowicz, State University of New York, Buffalo, N. Y. NZB mice were obtained from Ms. Jean Eastcott, Boston University School of Medicine, Boston, Mass. BALB/cBy +/+ and *nu/nu* mice were obtained from Sprague-Dawley Laboratories, Madison, Wis., and bred in our animal facility. Mice of both sexes were used and, where not indicated, were 6–8 wk old.

Tumor Cells. EL-4 (*H-2^b*) lymphoma cells obtained originally from Dr. John Mannick, Department of Surgery, Boston University School of Medicine, are maintained by serial passage in vivo by injecting $1-5 \times 10^6$ cells i.p. in B₆ mice. The in vitro EL-4 cell line is derived from the in vivo cell line by culturing in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.), with 10% fetal calf serum, penicillin and streptomycin, L-glutamine, sodium pyruvate, and nonessential amino acids (complete RPMI-1640 medium). Tissue-culture-maintained EL-4 cells become sensitive to NK activity within 4–6 wk. Before this time, the cells have a very high spontaneous release. Cells at this period in their passage history are frozen and stored in liquid nitrogen. This is necessitated because after $\cong 24$ wk in vitro, the genetic specificities reported in Table I tend to get lost. YAC-1 (*H-2^a*) lymphoma cells were obtained as a tissue-culture-adapted line from Dr. G. Cudkowicz and have been maintained in complete RPMI-1640 medium plus 10% fetal calf serum. Tissue-cultured RL δ 1 (*H-2^d*), a radiation-induced lymphoma of BALB/c mice was obtained from Dr. Howard Holden of the National Cancer Institute, Bethesda, Md. L1210 (*H-2^d*) was obtained from Dr. Hugues J.-P. Ryser of the Boston University School of Medicine.

Preparation of Lymphoid Cell Suspensions. This has been described previously (13).

NK-Cell Assay. 2×10^4 tumor cells in 0.1 ml of RPMI-1640 medium were labeled with ^{51}Cr (New England Nuclear, Boston, Mass.) and placed in wells of Microtest II plates (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) with varying numbers of freshly harvested lymphoid cells in 0.1 ml of complete RPMI-1640 medium. Generally, each spleen-cell suspension was plated at four different effector to target-cell (E:T) ratios ranging from 100:1 to 12.5:1. Cells at each ratio were plated in triplicate. In the case of EL-4 cells, the effectors and targets were incubated for 18 h before the supernatant fluids were collected by Titertek supernate collection system (Flow Laboratories, Inc. Rockville, Md.). When YAC-1 or RL δ 1

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were used as targets, the incubation period was four h. The EL-4 cells are not lysed in the NK assay at 4 h. The ^{51}Cr radioactivity in the supernatant fluids was counted in a well-type γ -scintillation counter. The mean percentage of specific cytotoxicity was calculated as follows: mean percentage of specific cytotoxicity = $(^{51}\text{Cr}$ cpm, experimental - ^{51}Cr cpm, spontaneous [medium]) / (^{51}Cr cpm, maximum [H_2O] - ^{51}Cr cpm, spontaneous) \times 100. The variation between the replicates was $<10\%$. Cold competition experiments were performed by fixing the E:T ratio at 50:1 (which is in the linear part of the curve) and the addition of varying numbers of cold (i.e., unlabeled) targets, generally at ratios of 10:1, 20:1, and 40:1; cold:hot (i.e., labeled). ^{51}Cr -release determinations with only labeled and cold targets were performed but they were never significantly different from spontaneous release values. The percentage of specific lysis of EL-4 cells by $\text{B}_6\text{D}_2\text{F}_1$ spleen cells varied from 9 to 28% in different experiments (E:T ratios of 50:1). Such variation in NK activity has been observed by others (1, 2). The specificity of the reaction at all levels of cytotoxicity was monitored by including $\text{B}_6\text{D}_2\text{F}_1$ thymus cells and B_6 spleen cells as effector cells in most assays. The cytotoxicity produced by these populations was always significantly lower ($P < 0.01$) than that produced by $\text{B}_6\text{D}_2\text{F}_1$ spleen cells.

Mouse Pretreatments. 6- to 8-wk-old mice were injected with two doses of 100 μCi ^{89}Sr (Oak Ridge National Laboratory, Oak Ridge, Tenn.) i.p. and utilized 3-6 wk after the last injection. The mortality among $\text{B}_6\text{D}_2\text{F}_1$ mice by this procedure is $\cong 10\%$. Silica particles (5- μm average size, gift from Dr. K. Robock, Steinkohlenberg Vauverein, Essen-Kray, West Germany) were suspended in medium, sonicated, and then injected i.v. (2.5 mg/mouse) or used in vitro (100 μg /well). *Corynebacterium parvum* was a gift of Dr. J. Hunter, Wellcome Laboratories, Raleigh, N. C. Mice were injected 2.1 mg (in 0.3 ml) i.p. Polyinosinic:polycytidylic acid (pI:pC) (Sigma Chemical Co., St. Louis, Mo.) was injected i.p. (0.12 mg/mouse). Cyclophosphamide (CY) was a gift from Mead Johnson & Co., Evansville, Ind.; mice were injected with 300 mg/kg body weight. Hydrocortisone acetate (Merck, Sharp & Dohme, West Point, Pa.) was injected i.p. (2.5 mg). Mice and cells were irradiated in a small-animal irradiator (Gammacell 40, Atomic Energy of Canada Ltd., Commercial Products, Ottawa, Ontario, Canada, dual ^{137}Cs sources, 136 rad/min).

Cell Pretreatments. Filtration over Sephadex G-10 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) columns was done by the method of Ly and Mishell (14). Yields of nonadherent cells varied between 40 and 50%. Treatment with anti-Ig and complement has been described (15). Treatment with anti-Thy-1.2 and complement was performed by the protocol described earlier. The anti-Thy-1.2 used is a monoclonal antibody derived from a hybridoma (New England Nuclear, Boston, Mass.). This preparation, which is presumably free of any contaminating anti-Ly activity, has a titer of $1:10^5$. In preliminary characterization studies, a 1:3,000 dilution of this preparation lysed 100% $\text{B}_6\text{D}_2\text{F}_1$ thymus cells, suppressed splenic concanavalin A (Con A) response by 90%, did not affect B-cell mitogenesis, and suppressed cytolytic T-cell activity completely. $\text{B}_6\text{D}_2\text{F}_1$ spleen cells were treated with a very high concentration of this preparation (1:500) before being tested in the NK assay.

Formation of Tumor-Cell and Other Monolayers, and Cell Adsorptions. Monolayers of tumor or other cells were prepared by some modification of previously described methods (16, 17). Essentially, poly-L-lysine (PLL) (50 $\mu\text{g}/\text{ml}$) was coated on plastic 60-mm Petri dishes (Falcon Labware, Div. of Becton, Dickinson & Co.) at room temperature for 1 h. After washing off the PLL with phosphate-buffered saline (PBS, pH 7.4), 2 ml of tumor-cell suspension ($40 \times 10^5/\text{ml}$) were plated on the dish and allowed to stay at room temperature for 45 min. Nonattached tumor cells were then poured off and the Petri dishes were rinsed with PBS. 40×10^6 spleen cells in complete RPMI-1640 medium ($20 \times 10^6/\text{ml}$) were poured on the tumor-coated dishes, and incubated at 37°C for 45 min. At the end, the nonadherent spleen cells were gently poured off and the dishes were washed once with 2 ml complete RPMI-1640 medium. The yield of nonadherent cells by this procedure was 30-40%. The contamination of nonadherent spleen cells by the tumor cells used to make monolayers was carefully monitored (on the basis of cell size) and was never $>7\%$. Monolayers with sheep erythrocytes (SE) and 7S antibody-coated sheep erythrocytes (SEA) were prepared exactly as described (18).

Generation of Anti-Parent Cytotoxic T Lymphocytes. A modification of the method described by Shearer and Cudkovic was used (6). 25×10^6 $\text{B}_6\text{D}_2\text{F}_1$ spleen cells were cultured with irradiated (1,000 rad) 25×10^6 B_6 spleen cells in complete RPMI-1640 medium. Cultures were performed in 25- cm^2 tissue-culture flasks (Corning Glass Works, Science Products Div., Corning, N. Y.)

incubated vertically for 5 d in a humidified 5% CO₂ incubator. At the end of 5 d, the cytotoxic cells so generated were incubated with ⁵¹Cr-labeled EL-4 cells in wells of Microtest II plates and the cytotoxicity was measured 4 h later. The collection of supernatant fluid and the calculations were performed as in the NK assay. The baseline controls were either tumor cells without any spleen cells or with B₆D₂F₁ spleen cells cultured with syngeneic irradiated spleen cells. The latter did not cause any significant release of ⁵¹Cr above the medium control.

Results

Strain Distribution of Good Responders to B₆ (H-2^b) Marrow Grafts Correlates with Ability of Spleen Cells to Lyse EL-4 but Not YAC-1 Target Cells. B₆D₂F₁, B10.D2, NZB, B10.A, and B10.A(5R) mice are able to reject B₆ or B10 marrow cells well and their spleen cells lysed EL-4 cells well in vitro (Table I). The opposite was also true, i.e., CBA, B₆, DBA/2, and A-strain mice are poor responders to B₆ or B10 marrow grafts and their spleen cells did not lyse EL-4 cells effectively. CBA and B₆ spleen cells lyse YAC-1 but not EL-4 cells well, whereas A-strain spleen cells lyse both targets poorly. These data extend the earlier findings with the EL-4 NK assay in that *Ir*-like gene functions against *Hh-1^b* antigens (4) are detected in addition to hybrid resistance (12).

Tissue Distributions of NK Activity against EL-4 Lymphoma. NK activity of lymphoid tissues from B₆ and B₆D₂F₁ mice against EL-4 and YAC-1 targets was compared (Table II). All B₆D₂F₁ tissues tested showed NK activity against EL-4 and, in each instance, F₁ cells killed the target better than B₆ parental cells. Lysis of EL-4 cells was maximal by spleen, lymph node cells, and peritoneal cells. Bone marrow was relatively poor. Peritoneal cells and bone-marrow cells had very little NK activity against YAC-1 as previously described (9, 10).

TABLE I
Strain Distribution of NK Activity against EL-4 and YAC-1 Tumor Cells

Mouse strain	Ability to reject B ₆ bone-marrow grafts*	Target cells	Percentage of mean cytotoxicity at E:T of		
			100:1	50:1	25:1
			%		
CBA	—	YAC-1‡	54.7	31.3	20.2
B ₆	—	YAC-1	46.2	29.6	17.4
B ₆ D ₂ F ₁	+ +	YAC-1	35.4	20.5	13.0
DBA/2	—	YAC-1	20.5	11.3	7.0
CBA	—	EL-4§	0.7	1.1	—0.8
B ₆	—	EL-4	—1.1	0.4	0.9
B ₆ D ₂ F ₁	+ +	EL-4	19.5	12.1	5.9
DBA/2	—	EL-4	0.4	1.7	—2.1
B10.D2	+ +	EL-4	14.5	9.3	5.0
NZB	+	EL-4	30.7	18.7	6.4
B10.A	+ +	EL-4	17.3	12.1	9.2
A	—	EL-4	4.2	1.0	1.5
B10.A(5R)	+	EL-4	12.5	8.4	3.2

* Data from references 1, 2, 19. Plus (+), good rejector of B₆ bone marrow, minus (—), poor rejector of B₆ bone marrow.

‡ YAC-1 target cells assayed in a 4-h ⁵¹Cr-release assay.

§ EL-4 target cells assayed in an 18-h ⁵¹Cr-release assay.

|| Unpublished observations of J. W. Eastcott and M. Bennett.

TABLE II
NK Activity in Different Lymphoid Tissues against EL-4 and YAC-1 Cells

Experiment	Lymphoid tissue	Strain of mice	Percentage of cytotoxicity*	
			EL-4‡	YAC-1§
1	Lymph node	B ₆ D ₂ F ₁	8.9	—
		B ₆	—11.7	—
	Bone marrow	B ₆ D ₂ F ₁	5.2	18.2
		B ₆	2.1	4.8
	Spleen	B ₆ D ₂ F ₁	13.3	44.6
		B ₆	3.4	34.0
2	Spleen	B ₆ D ₂ F ₁	22.5	39.5
	Peritoneal cells	B ₆ D ₂ F ₁	16.5	10.3

* 50:1 E:T ratio.

‡ See Table I.

§ See Table I.

Characterization of the Anti-EL-4 NK Cells. NK activity against EL-4 cells was present in spleen-cell suspensions of BALB/c *nu/nu* mice and was not sensitive to treatment with rabbit anti-mouse Ig and complement (Table III). Filtration of spleen cells over Sephadex G-10 columns and adhesion to plastic did not remove the NK cells. These features suggest that the cells are similar to those described for other NK cells (9, 10). However, unlike the NK cells against YAC-1 (10) and RL δ 1 targets, the anti-EL-4 NK cells were not affected by injection of mice with silica or incubation with silica particles in vitro. Furthermore, anti-EL-4 NK cells could not be adsorbed by monolayers of SEA (18). Thus, anti-EL-4 NK cells do not bear Fc receptors. The efficacy of the removal of Fc⁺ cells in the same experiment was ascertained by testing antibody-dependent cellular cytotoxicity (ADCC) against antibody-coated chicken erythrocytes. ADCC was reduced by 80%. In our hands, adsorption over SEA monolayers consistently enriches for NK activity against EL-4. Pretreatment of cells with a high-titered monoclonal anti-Thy-1.2 preparation and complement did not affect the NK activity against EL-4 cells. However, the very same anti-Thy-1.2-treated cells showed a 25–37% reduction in NK activity against YAC-1 cells.

NK Activity against EL-4, YAC-1, and RL δ 1 Targets in Spleens of ⁸⁹Sr-treated Mice. Lysis of EL-4 cells was normal in 7 out of 10 experiments performed at different times, using pooled spleen cells from two ⁸⁹Sr-treated B₆D₂F₁ mice in each experiment (Table IV). In the other three experiments, lysis of EL-4 cells by spleen cells from ⁸⁹Sr-treated mice was reduced by \cong 70%. On the other hand, NK activity against YAC-1 and RL δ 1 was consistently reduced by 70–80% in ⁸⁹Sr-treated mice. NK activity against YAC-1 is detected routinely in a 4-h assay and 18 h is required for lysis of EL-4. It was therefore important to ascertain if normal lysis of EL-4 target by spleen cells of ⁸⁹Sr-treated mice was not an artifact produced by a longer incubation time. To this end, we performed the anti-YAC-1 NK assay for 18 h, and still failed to observe lysis by spleen cells from ⁸⁹Sr-treated mice. The converse, i.e., 4-h anti-EL-4 assay, cannot be performed because spleen cells from normal mice fail to lyse EL-4 cells as rapidly.

TABLE III
NK Activity against EL-4 Cells: Characterization of Effector Cells in the Spleen

Experiment	Mice	Cell pretreatment	Percentage of cytotoxicity*
			%
1	BALB/cBy +/+	—	8.6
	BALB/cBy nu/nu	—	12.0
2	B ₆ D ₂ F ₁	—	10.9
		Normal rabbit serum + complement	11.0
		Anti-Ig + complement	27.0
3	B ₆ D ₂ F ₁	—	28.1
		Filtered over Sephadex G-10	22.4
		Nonadherent to plastic	32.5
4	B ₆ D ₂ F ₁	—	19.6
		Silica in vivo‡	21.9
5	B ₆ D ₂ F ₁	—	24.3
		Silica in vitro (preincubation)‡	27.8
		Silica in vitro (in the assay)‡	30.5
6	B ₆ D ₂ F ₁	—	13.1
		SE monolayer§	22.3
		SEA monolayer	27.4
7	B ₆ D ₂ F ₁	—	32.7
		Complement	33.1
		Anti-Thy-1.2 + complement	36.6

* Mean percentage of cytotoxicity at an E:T ratio of 50:1.

‡ Mice were injected with 2.5 mg of silica particles i.v. 24 h before sacrifice in experiment 4; in experiment 5, 20×10^6 spleen cells were incubated with 100 μ g of silica for 45 min at 37°C, washed once, and then tested; or an equal amount of silica was allowed to stay in the cell suspension during the 18-h assay period.

§ Monolayers of SE or SEA were prepared on Petri dishes as described in Methods. Cells nonadherent to the monolayers after 45-min incubation at 37°C were used. Cells nonadherent to SEA monolayers were tested for ADCC against chicken erythrocytes. ADCC was reduced from 35 (control cells) to 5%.

|| Cytotoxicity against YAC-1 targets in the same experiment: no treatment, 24.9%; complement alone, 25.3%; and anti-Thy-1.2 + complement, 18.4%.

NK Activity in Neonatal and Aging Mice. In contrast to NK cells for YAC-1 targets, NK-cell activity against EL-4 targets was demonstrable in B₆D₂F₁ and BALB/cBy nu/nu mice by 5 and 7 d of age respectively (Table V). Moreover, the loss of NK activity against YAC-1 cells associated with ages >12 wk (9, 10) did not occur for NK cells capable of lysing EL-4 cells.

Effect of Filtration of Spleen Cells over Sephadex G-10 Columns. In ⁸⁹Sr-treated mice, the NK activity against EL-4 cells was reduced in only 30% of the experiments (Table IV). In two of those instances, spleen cells of control and ⁸⁹Sr-treated mice were filtered over Sephadex G-10 columns. Sephadex G-10 filtration restored NK-cell activity against EL-4 cells but not against YAC-1 cells (Table VI). The data suggested that anti-EL-4 NK cells are present in ⁸⁹Sr-treated mice and that suppressor cells adherent to Sephadex G-10 may be responsible for the occasional low NK values.

TABLE IV
Effect of ^{89}Sr Treatment on NK Activity of $B_6D_2F_1$ Spleen Cells

Target cells	Pretreatment of spleen cell donors		Percentage of control values
	None (percentage of cytotoxicity)	$^{89}\text{Sr}^*$ (percentage of cytotoxicity)	
	%		%
EL-4‡	16.7	16.2	97
	11.5	17.9	159
	27.5	21.3	77
	18.9	20.1	106
	14.3	13.2	92
	23.5	20.9	88
	18.2	19.5	107
			(103.7 ± 9.3)§
	17.1	5.3	30
	19.7	6.5	32
	26.3	6.1	23
		(28.3 ± 2.2)§	
YAC-1	45.8	3.1	7
	35.6	6.2	17
	30.4	7.8	25
	29.1	6.5	22
	31.5	5.2	16
		(17.4 ± 2.7)§	
RLδ1	15.9	3.1	20

* Donors of spleen cells injected with ^{89}Sr (100 μCi) i.p. on two occasions, 4 wk apart. Mice used 4–7 wk after the last injection.

‡ The percentage of specific release of ^{51}Cr measured at E:T ratios of 50:1; incubation period was 18 h with EL-4 cells and 4 h with YAC-1 and RLδ1 cells except where indicated.

§ Represents the average (± SEM) of the preceding group of the percentages of the control values.

|| In this experiment YAC-1 cells were incubated for 18 h.

TABLE V
Age-dependent Changes in NK-Cell Functions

Experiment	Age of spleen cell donors	Mouse strain	Mean percentage of specific cytotoxicity	
			YAC-1	EL-4
			%	
1	5 d	$B_6D_2F_1$	5.7*	14.5
	16 d	$B_6D_2F_1$	7.9	12.3
	30 d	$B_6D_2F_1$	35.1	16.7
	18 mo	$B_6D_2F_1$	9.3	18.5
2	7 d	BALB/c nu/nu	—	13.2
	40 d	BALB/c nu/nu	—	12.0

* 50:1 E:T ratio; incubation periods of 4 (YAC-1) or 18 (EL-4) h.

Adoptive Transfer of NK-Cell Activity. B₆D₂F₁ mice were irradiated and repopulated with inocula of 1×10^6 marrow cells or 6×10^6 spleen cells from syngeneic donor mice so as to provide equivalent numbers of hemopoietic colony-forming cells (20). Recipient spleens tested at different intervals after cell transfer were inactive for NK-cell function against EL-4 and YAC-1 targets on day 7 (Table VII). NK cells against EL-4 targets were generated to near-normal levels by both spleen and marrow precursors by day 14. Marrow cells generated NK cells for YAC-1 targets more rapidly than did spleen cells. Such data are consistent with the concepts of separate NK-cell lines or two separate stages in differentiation of a common cell line (the anti-EL-4 cells preceding the anti-YAC-1 cell).

Effects of CY, Hydrocortisone Acetate, C. parvum, and pI:pC on NK-Cell Function. These agents had similar effects on both types of NK cells (Table VIII). Suppression was detected 3 d after injection of CY or hydrocortisone acetate and 13 d after injection of *C. parvum*. Stimulation was observed 1 d after injection of pI:pC, 3 d after injection of *C. parvum*, and 13 d after injection of CY.

Ability of Unlabeled EL-4 and YAC-1 Cells to Inhibit NK-Cell Activity. To detect any cross-reacting antigens on EL-4 and YAC-1 target cells, we performed NK-cell assays at a 50:1 E:T ratio and added varying numbers of cold (unlabeled) EL-4 or YAC-1 cells at ratio known to inhibit cytotoxicity. We observed extensive inhibition of cytotoxicity toward ⁵¹Cr-labeled YAC-1 cells by cold YAC-1 and cold EL-4 cells (Table IX). The same was true for ⁵¹Cr-labeled EL-4 cells. These data indicate that both types of NK cells recognize both types of lymphoma cells, despite differences in the nature of the two cells and the time required for lysis in the two assays.

Adsorption of Anti-EL-4 and Anti-YAC-1 NK Activity by EL-4 and YAC-1 Monolayers. It was important to verify the conclusions arrived at by cold competition by an independent approach. We prepared monolayers of YAC-1 and EL-4 cells on PLL-coated Petri dishes. Spleen cells were then incubated for 45 min at 37°C on each of the two monolayers. Following this, the nonadherent spleen cells from each monolayer

TABLE VI
Effect of Sephadex G-10 Filtration on the NK Activity of Spleen Cells of ⁸⁹Sr-treated B₆D₂F₁ Mice

Experiment	Pretreatment of spleen cell donor	Sephadex G-10 filtration of cells	Percentage of cytotoxicity of NK target cells*	
			EL-4	YAC-1
1	Controls	No	19.7	32.6
		Yes	25.6	41.5
	⁸⁹ Sr‡	No	6.5	5.2
		Yes	30.4	7.9
2	Controls	No	21.8	18.5
		Yes	32.1	22.3
	⁸⁹ Sr‡	No	17.6	6.5
		Yes	21.2	9.4

* Mean percentage of specific cytotoxicity at 50:1 E:T ratio. Incubation periods 18 h (EL-4) or 4 h (YAC-1).

‡ 100 μCi i.p. twice at monthly intervals; tested 4–7 wk after second injection.

TABLE VII
NK Activity of Spleen Cells in Lethally Irradiated B₆D₂F₁ Mice Reconstituted by Normal Spleen Cells or Bone-Marrow Cells

Cell type*	Days after cell transfer	Percentage of cytotoxicity of NK target‡	
		EL-4	YAC-1
		%	
Bone marrow	7	5.7 (31.8)§	7.8 (26.4)
	14	14.9 (94.9)	21.7 (67.6)
	21	19.2 (112)	35.2 (94.1)
Spleen	7	6.2 (34.6)	4.6 (15.5)
	14	19.4 (123)	7.2 (22.4)
	21	15.6 (91.2)	14.1 (37.7)
	30	22.7 (119)	35.2 (117)

* 1×10^6 bone-marrow cells or 6×10^6 spleen cells were infused into lethally irradiated (850 rad) B₆D₂F₁ mice.

‡ Mean percentage of cytotoxicity at an E:T ratio of 50:1.

§ Numbers in parentheses represent the cytotoxicity of spleen cells from reconstituted mice expressed as the percentage of the cytotoxicity observed in normal spleen cells, tested in parallel.

TABLE VIII
Effect of Various Immunoregulatory Agents on NK Activity of B₆D₂F₁ Spleen Cells against YAC-1 and EL-4 Cells

Experiment	Donor pretreatment*	Days after treatment	Mean percentage of cytotoxicity of NK target cells‡	
			EL-4	YAC-1
			%	
1	—	—	19.4	36.5
	CY	3	2.1	15.7
	<i>C. parvum</i>	3	39.4	56.4
	Hydrocortisone acetate	3	4.2	14.9
2	—	—	14.5	31.5
	CY	13	25.6	55.8
	<i>C. parvum</i>	13	4.5	6.2
3	—	—	10.0	31.5
	pI:pC	1	41.7	49.5

* Mice were injected i.p. with 300 mg/kg of CY, 2.1 mg *C. parvum*, 2.5 mg of hydrocortisone acetate, or 0.12 mg of pI:pC.

‡ Mean percentage of cytotoxicity at an E:T ratio of 50:1.

were gently eluted and tested for NK activity on EL-4 and YAC-1 targets. It was reasoned that if YAC-1 and EL-4 cells shared some common antigen and if the anti-YAC-1 and anti-EL-4 cells shared receptor structures for the common antigens, adsorption of cells over either of the two monolayers would simultaneously deplete NK activity against both the targets. Nonadherent cells recovered from YAC-1 or EL-4 monolayers were almost completely depleted of NK activity against both the targets (Table X). Adsorption of spleen cells over Petri dishes coated with PLL only,

TABLE IX
Competitive Inhibition of NK Activity against EL-4 and YAC-1 Cells

Cold cell type	Percentage of inhibition of cytotoxicity against:*					
	EL-4 (cold:hot)			YAC-1 (cold:hot)		
	10:1	20:1	40:1	10:1	20:1	40:1
	%					
EL-4	22	64	89	19	57	75
YAC-1	34	69	91	44	75	99

* 100% cytotoxicity against EL-4 and YAC-1 (i.e., without cold targets) was 19.5 and 39.7%, respectively.

TABLE X
Adsorption of NK Activity against EL-4 and YAC-1 Cells by EL-4 and YAC-1 Monolayers

Experiment	Adsorption of B ₆ D ₂ F ₁ spleen cells on	Percentage of specific cytotoxicity for*	
		EL-4	YAC-1
		%	
1	—	—	51.5
	PLL only	—	50.6
	B ₆ D ₂ F ₁ thymus cells	—	44.6
	YAC-1	—	5.3
	EL-4	—	10.1
2	—	28.5	34.6
	SE	29.7	38.4
	YAC-1	5.3	8.4
	EL-4	3.2	5.9
	L1210	25.9	31.7

* Mean value for 50:1 E:T ratio.

B₆D₂F₁ thymus cells, SE, or DBA/2 L1210 tumor cells did not affect NK activity against either target, indicating a specificity for NK cells. A possible source of error in such experiments can arise from detachment of tumor cells forming the monolayers during the elution of nonadherent cells. The tumor cells mixed with the eluted cells could then act as cold competitors in the next step of the assay. We carefully monitored for such contamination (Methods) and found that detached tumor cells constituted $\cong 5\%$ of the nonadherent population. Using an E:T ratio of 50:1, this degree of contamination would result in a cold:hot target ratio of $\cong 2:1$, which is far below that required to produce significant cold competition (Table IX).

Lysis of EL-4 and YAC-1 cells by B₆D₂F₁-Anti-B₆ Effector Cells Induced In Vitro. B₆D₂F₁-anti-B₆ cytotoxic T cells were generated in a cell-mediated lympholysis (CML) system (6). Irradiated B₆ (*H-2^b*) spleen cells were the stimulators and the ⁵¹Cr-labeled target cells included EL-4 (*H-2^b*), YAC-1 (*H-2^a*), and L1210 (*H-2^a*) tumor cells. The effector cells presumably recognized *Hh-1^b* determinants present on normal *H-2^b* hemopoietic cells (6). Both EL-4 and YAC-1 target cells were lysed by the F₁-anti-parent effector cells (Table XI). The L1210 tumor cells, which are very poor targets for NK cells, were not lysed by these F₁-anti-parent cytotoxic T cells.

TABLE XI
Lysis of EL-4 and YAC-1 Cells by B₆D₂F₁ Anti-B₆ Cytolytic T Cells

Target cell	Mean percentage of specific cytotoxicity at E:T ratios		
	50:1	25:1	12.5:1
	%		
EL-4	30.5	21.2	8.9
YAC-1	24.7	16.5	7.1
L1210	5.1	2.7	2.0

Discussion

Genetic resistance to hemopoietic allografts and NK-cell activity against hemopoietic tumors are two unusual phenomena that appear to have several similarities (10). However, the exact relationship between the effector cells responsible for the two activities is not entirely clear. Although several studies of NK cells have appeared recently, the interrelationship between NK cells active against various tumor target cells has not been elucidated. The studies reported here addressed this problem.

NK activity against tissue culture-adapted EL-4 cells has several features that distinguish it from NK activity against YAC-1 and RL δ 1 cells, two target cells widely used to investigate NK cells. NK cells for YAC-1 (10, 11) or RL δ 1 cells (Table IV) and viral-induced NK cells (21) are depleted in mice treated with ⁸⁹Sr, indicating that such NK cells require an intact marrow microenvironment for some critical stage in differentiation. Thus these cells are similar to M cells which have been previously suggested to be effector cells responsible for marrow allograft reactivity (5), genetic resistance to the leukemic and immunosuppressive effects of Friend virus (22), resistance to infection with *Listeria monocytogenes* (23), and genetic resistance to encephalomyelitis induced by herpes simplex virus-1 (24). However, because we have also observed an increase in thymus-dependent and thymus-independent suppressor cells in spleens of mice treated with ⁸⁹Sr (15, 25), the possibility that poor NK-cell function in ⁸⁹Sr-treated mice is due to suppressor cells rather than to depletion of effector M cells must be considered. We feel this is unlikely because we have been unable to detect any suppressor cells for anti-YAC-1 NK activity by a variety of techniques previously utilized to detect suppressor cells in ⁸⁹Sr-treated mice (15, 25).³

It should be pointed out that the effects of ⁸⁹Sr administration on the lymphoid system are fairly specific. After ⁸⁹Sr treatment, several thymus-dependent-cell, bursa equivalent-dependent-cell, and macrophage functions and/or numbers assessed by a variety of assays remain normal (26). Study of ⁸⁹Sr-treated mice allows the distinction to be made between those immune cells that are normally marrow-derived but not M (e.g., T cells, B cells, macrophages) and those which are not only marrow-derived but are also M cells (e.g., anti-YAC-1 NK cells). In ⁸⁹Sr-treated mice the spleen takes over stem-cell functions and the marrow-independent cells can differentiate normally.

In contrast to NK cell-activity against YAC-1 and similar targets discussed above, the NK-cell activity against EL-4 cells was not usually affected by treatment of mice with ⁸⁹Sr. In the few instances where NK activity against EL-4 cells was low in ⁸⁹Sr-

³ Kumar, V., J. Ben-Ezra, M. Bennett, and G. Sonnenfeld. Natural killer cells in the mice treated with ⁸⁹Sr: normal target-binding cell numbers but inability to kill even after interferon administration. *J. Immunol.* In press.

treated mice, the effect was apparently due to presence of suppressor cells rather than to a lack of EL-4 NK cells, because the low NK activity against EL-4 could be restored to normal by filtration over Sephadex G-10 columns.² (Also, Table VI.) Note that in the same experiment, the low NK activity against YAC-1 could not be reversed by filtration over Sephadex G-10 columns. To our knowledge, this is the first demonstration of two biologically (or developmentally) distinct classes of NK cells in mice: (a) M cells, and (b) marrow-independent (non-M) cells. Further support (but not proof) for such a conclusion comes from the comparative study of ontogeny of NK cells and their generation in irradiated recipients. M-cell activity as measured by marrow allograft reactivity (1) and resistance against Friend virus leukemia (13) does not develop until mice are 3 wk old; similarly, NK-cell activity against YAC-1 cells does not develop until mice are \cong 18–20 d old (Table V, and [10]). On the other hand, normal NK-cell activity against EL-4 cells is present as early as 5 d after birth. In adoptive transfer experiments, it has been seen that M-cell functions (marrow allograft reactivity, early resistance against *Listeria monocytogenes*) are more efficiently generated in irradiated syngeneic recipients by bone-marrow cells than by spleen cells (27, 28). This could be explained by the higher frequency of M-cell precursors in the bone marrow, as compared with the spleen. Correspondingly, the NK activity against YAC-1 cells was much more rapidly generated in irradiated mice repopulated with bone-marrow cells, as compared with recipients of normal spleen cells, even though six times as many spleen cells were transplanted to correct for the relative frequencies of the pluripotent stem cells. In contrast, NK-cell activity against EL-4 cells was generated within 15 d after transfer of spleen or bone-marrow cells. Another distinction between anti-YAC-1 and anti-EL-4 cells is the susceptibility to anti-Thy-1.2 and complement. NK activity against EL-4 cells is not affected by pretreatment of the cells with a high-titered monoclonal anti-Thy-1.2 and complement, whereas, in agreement with the observation of others, the NK activity against YAC-1 cells is reduced by \cong 30% (29).

It must be pointed out that there are also certain similarities between the anti-YAC-1 and anti-EL-4 NK cells. Both the activities are stimulated 3 d after injection of *C. parvum* and suppressed 13 d after *C. parvum* injection or by hydrocortisone acetate and CY. As discussed later in this paper, these findings are consistent with the notion that anti-EL-4 NK cells may be the precursors of anti-YAC-1 NK cells.

The nature of the target structures on most NK-sensitive tumor cells is still not clear. However, in the case of tissue-culture-adapted EL-4 cells, it seems very likely that the critical target antigen is the same as, or similar to, the *Hh-1^b* antigen. This antigen is expressed on primitive hemopoietic cells of the C57BL/6 (*H-2^b*) mice and appears to be the relevant antigen in the rejection of B₆ bone-marrow cells by irradiated mice. The *Hh-1* locus has been mapped at, or close to, the D-end of *H-2* complex and is not expressed in the heterozygous state, resulting in the rejection of B₆ bone-marrow cells by B₆D₂F₁ hybrid mice (hybrid resistance) (3). Our suggestion that *Hh-1^b* antigen is the critical target structure in the lysis of EL-4 cells in the NK assay is based on the strong immunogenetic correlation between NK activity against EL-4 cells in vitro and rejection of B₆ bone-marrow grafts in vivo. This can be summarized as follows: (a) hybrid resistance is seen against B₆ bone-marrow grafts in vivo and in the NK-cell activity against EL-4 in vitro. This phenomenon cannot be due to dominant *Ir*-like genes inherited from the opposite parent, because the DBA/2 parent

itself is both a poor responder to B₆ bone-marrow allografts in vivo and shows poor NK activity against EL-4 cells in vitro (Table I). Furthermore, when spleen cells from a number of F₁ mice derived from intra-*H-2* recombinant parents were tested (12), it was found that heterozygosity at the D-end of *H-2* was critical for lysis of EL-4 cells in the NK assay. Heterozygosity at other parts of the *H-2* complex was neither necessary nor sufficient for hybrid resistance in the NK assay. The fine genetics of hybrid resistance against parent *Hh-1^b* antigens in vivo is similar to that seen in the NK assay, i.e., only those F₁ mice that are heterozygous at *H-2D^b* reject B₆ bone-marrow cells; (b) among inbred strains, reactivity against EL-4 in vitro and B₆ bone-marrow grafts in vivo is regulated by non-*H-2* genes on the C57BL background. For example, both DBA/2 and B10.D2 are *Hh-1* incompatible with B₆, but B10.D2 mice are good rejectors of B₆ bone-marrow grafts, whereas DBA/2 mice are poor responders (30). The anti-EL-4 NK reactivity of spleen cells from DBA/2 and B10.D2 mice follows the same pattern. Other such examples can be seen in Table I.

Because the genetic pattern of NK reactivity against the YAC-1 does not correspond to the anti-EL-4 NK reactivity or anti-B₆ (*Hh-1^b*) bone-marrow reactivity in vivo, it appears unlikely that the lysis of YAC-1 cells is mediated solely by recognition of *Hh-1^b* antigens in vitro. However, cold competition experiments and adsorption experiments (Tables IX and X) compel us to conclude that YAC-1 and EL-4 cells must share some antigen critical for the lysis of both the targets. To resolve whether *Hh-1^b* or some other antigen (e.g., putative NK antigens) is shared by YAC-1 and EL-4 cells, we tested the ability of B₆D₂F₁ anti-B₆ cytotoxic T lymphocytes to lyse EL-4 and YAC-1 target cells. Such anti-*Hh-1^b* cytotoxic T lymphocytes lysed EL-4 cells and YAC-1 cells with equal efficiency (Table XI). Lysis of EL-4 cells could be inhibited by cold YAC-1 cells and vice versa. Detailed analysis of such cross-reactive lysis⁴ has shown that the lysis of YAC-1 cells under these conditions is not due to anti-fetal calf serum activity or persistence of NK cells of the responder spleen cells during culture. The anti-*Hh-1^b* effector T cells do not lyse normal A-strain cells (Con A blasts). This rules out the nonspecific activation of anti-*H-2^a* clones as the reason for cross-reactive lysis. Other possible explanations of cross-reactive lysis seen in some allogeneic CML reactions, such as sharing of public antigens (31), is rendered unlikely by the fact that *H-2*-associated public antigens are codominantly inherited and therefore no cytotoxicity against classical *H-2* antigens is likely to be generated in an F₁ anti-parent reaction. These data support the notion that YAC-1 cells express *Hh-1^b* antigens. Expression of inappropriate histocompatibility antigens on tumor cells has also been suggested by other studies (32).

The presence of *Hh-1^b* antigens on the YAC-1 cells may explain the cold competition between YAC-1 and EL-4 cells. However, two other observations also need explanation: (a) the poor lysis of YAC-1 cells by spleen cells of ⁸⁹Sr-treated mice, which seem to possess normal NK activity against *Hh-1^b*-positive EL-4 cells; (b) the lack of genetic correlation between the lysis of EL-4 and YAC-1 cells. Two models, both of which are consistent with most of the observed phenomena, are presented in Fig. 1. Model I proposes that YAC-1 and EL-4 are lysed by two different subsets of NK cells, and that the anti-YAC-1 NK cell is an M cell. EL-4 cells bear *Hh-1^b* antigens and the lysis

⁴ Thomas, A., V. Kumar, and M. Bennett. Hemopoietic histocompatibility antigens as possible targets for NK cells. Manuscript in preparation.

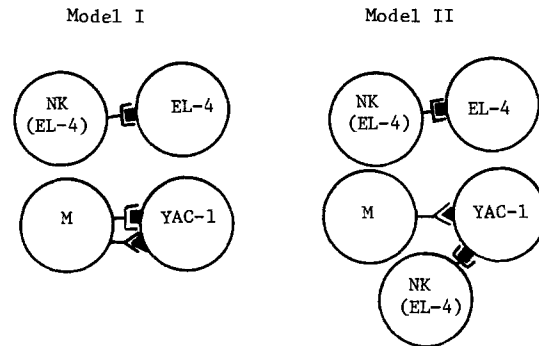


FIG. 1. Two models to explain NK-cell activity against EL-4 and YAC-1 tumor cells. (■) represents the *Hh-1^b* antigen, (▲) represents the NK antigen.

of the EL-4 cells involves interaction with anti-*Hh-1^b* receptors present on the NK cell. The lysis of YAC-1 cells requires recognition of *Hh-1^b* antigen as well as simultaneous recognition of one or more other determinants. Because both types of NK cells would have an anti-*Hh-1^b* receptor, cold competition and adsorption by monolayers would be explained. Anti-EL-4 NK cells could bind the YAC-1 cells, but in the absence of the other receptor, could not lyse them. Because binding and cell lysis are two discrete and independent steps in the NK system such a situation is quite conceivable (33). It is also possible that anti-EL-4 cells can lyse the YAC-1 cells, albeit inefficiently. The low anti-YAC-1 cytotoxicity seen in spleen cells of ⁸⁹Sr-treated mice could be a manifestation of the above. Similarly, anti-YAC-1 NK cells (M cells) would be expected to be able to bind EL-4 cells. However, it is difficult to test whether M cells can or cannot lyse EL-4 targets, because we do not have any physical method at present of selectively depleting the NK activity against EL-4 cells from normal spleen-cell suspensions. It is possible that anti-EL-4 NK cells are precursors of anti-YAC-1 cells. This differentiation of NK cells would require the marrow microenvironment. Model II differs from the previous one only in one important aspect: i.e., the lysis of YAC-1 requires participation of two cells. One of the two cells is a non-M cell and the same as EL-4 NK cell, and the other is an M cell. This model, like the previous one, can explain the effect of ⁸⁹Sr treatment, the cold competition and adsorption data, as well as the presence of normal frequency of YAC-1-binding cells in spleens of ⁸⁹Sr-treated mice.³ Experiments to distinguish between these two models are in progress.

One of the objectives of our work has been to develop an *in vitro* assay that would reflect all aspects of *in vivo* marrow allograft reactivity. The NK activity against EL-4 fulfills most of the immunogenetic requirements for such an assay, but seems not to reflect the *in vivo* situation entirely because the anti-EL-4 activity is retained in ⁸⁹Sr-treated mice. On the other hand, the anti-YAC-1 NK reactivity parallels many facets of marrow graft rejection except for immunogenetic specificity. It is tempting to speculate that both M cells and non-M cells are required for marrow allograft reactivity *in vivo* and that the complex effector-cell system has been dissected *in vitro*.

Summary

Natural killer (NK) cells from nonimmunized mice capable of lysing EL-4 (C57BL/6 strain *H-2^b*) tissue culture-adapted lymphoma cells have been analyzed and

compared with NK cells which lyse YAC-1 (A-strain, $H-2^a$) lymphoma cells. A correlation was seen in the ability of inbred and $B_6D_2F_1$ mice to reject C57BL/6 (B_6) bone-marrow grafts and the ability of their spleen cells to lyse EL-4 cells *in vitro*. This suggests that hybrid or hemopoietic histocompatibility antigens, ($Hh-1^b$), relevant in the rejection of B_6 stem cells may also be the relevant target structures for the anti-EL-4 NK cells. Certain features of these NK cells are similar to the NK cells reactive against YAC-1 cells. Both types of NK cells are present in athymic nude mice, are not affected by treatment with anti-immunoglobulin plus complement, and are not depleted by techniques that remove macrophages. NK activity against both targets is stimulated 3 d after injection of *Corynebacterium parvum*, and 24 h after challenge with polyinosinic:polycytidylic acid. Hydrocortisone acetate and cyclophosphamide lead to reduction of NK activity within 2–3 d after administration. However, the anti-YAC and anti-EL-4 NK reactivities differed in several important respects. Treatment of mice with ^{89}Sr , the bone-seeking isotope, to deplete marrow-dependent cells, depleted the anti-YAC-1 but not anti-EL-4 cell functions. Anti-EL-4 NK cells were unaffected by silica particles *in vivo* or *in vitro*; the NK cells reactive to EL-4 cells matured functionally much earlier in life (5 d of age) and the function did not decline with age. Irradiated mice reconstituted with syngeneic marrow or spleen cells developed functional NK cells against EL-4 targets before they developed anti-YAC-1 NK cells in their spleen. Thus anti-EL-4 NK cells that express hybrid resistance *in vitro* appear to differ from anti-YAC-1 NK cells and do not require an intact marrow microenvironment for functional differentiation.

Despite differences in the NK-cell types involved in the lysis of YAC-1 and EL-4 cells, these two tumor cells share certain common determinants. This was ascertained both by cold competition and by utilization of YAC-1 and EL-4 cell monolayers as immunoadsorbents. We conclude that $Hh-1^b$ is the common antigen present in EL-4 and YAC-1 cells, because $B_6D_2F_1$ anti- B_6 (anti- $Hh-1^b$) cytotoxic T lymphocytes lysed both the tumor cells. Our data suggests that $Hh-1^b$ antigen is recognized by both types of NK cells, but that additional determinants must be present on YAC-1 cells. Two models of NK cell lysis compatible with the data are presented.

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References

1. Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. I. Graft rejection by irradiated responder mice. *J. Exp. Med.* **134**:83.
2. Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant F_1 hybrid mice. *J. Exp. Med.* **134**:1513.
3. Cudkowicz, G. 1971. Genetic regulation of bone marrow allograft rejection in mice. *In Cellular Interactions in Immune Response*. S. Cohen, G. Cudkowicz, and R. T. McCluskey, editors. S. Krager, Basel, Switzerland. 93.
4. Cudkowicz, G. 1971. Genetic control of bone marrow graft rejection. I. Determinant specific difference of reactivity in two pairs of inbred mice strains. *J. Exp. Med.* **134**:281.
5. Bennett, M. 1973. Prevention of marrow allograft rejection with radioactive strontium: evidence for marrow dependent effector cells. *J. Immunol.* **110**:510.
6. Shearer, G. M., and G. Cudkowicz. 1975. Induction of F_1 hybrid anti-parent cytotoxic effector cells: an *in vitro* model for hemopoietic histocompatibility. *Science (Wash. D. C.)*. **190**:890.
7. Shearer, G. M., G. Cudkowicz, A. M. Schmitt-Verhulst, T. G. Rehn, H. Waksal, and P. D.

- Evans. 1977. F₁ hybrid anti-parent cell mediated lympholysis: a comparison with bone marrow graft rejection and with cell-mediated lympholysis to allo-antigens. *Cold Spring Harbour Symp. Quant. Biol.* **41**:511.
8. Herberman, R. B., M. F. Nunn, and D. H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterisation of effector cells. *Int. J. Cancer.* **16**:230.
 9. Kiessling, R. E., E. Klein, H. Pross, and H. Wigzell. 1975. Natural killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *Eur. J. Immunol.* **5**:117.
 10. Kiessling, R., P. S. Hochman, O. Haller, G. M. Shearer, H. Wigzell, and G. Cudkowicz. 1977. Evidence for a similar or common mechanism for natural killer cell activity and resistance to hemopoietic grafts. *Eur. J. Immunol.* **7**:655.
 11. Haller, O., and H. Wigzell. 1977. Suppression of natural killer activity with radioactive strontium: effector cells are marrow-dependent. *J. Immunol.* **118**:1503.
 12. Harmon, R. C., E. A. Clarke, C. O'Toole, and L. S. Wixler, 1977. Resistance of H-2 heterozygous mice to parental tumors. I. Hybrid resistance and natural cytotoxicity to EL-4 are controlled by the H-2D Hb-1 region. *Immunogenetics.* **4**:601.
 13. Kumar, V., and M. Bennett. 1976. Mechanisms of genetic resistance to Friend virus leukemia in mice. II. Resistance of mitogen responsive lymphocytes mediated by marrow-dependent cells. *J. Exp. Med.* **143**:713.
 14. Ly, I. A., and R. I. Mishell. 1974. Separation of mouse spleen cells by passage through columns of Sephadex G-10. *J. Immunol. Methods.* **5**:239.
 15. Kumar, V., T. Caruso, and M. Bennett. 1976. Mechanisms of genetic resistance to Friend virus leukemia. III. Susceptibility of mitogen responsive lymphocytes mediated by T cells. *J. Exp. Med.* **143**:728.
 16. Kedar, E., M. Ortiz de Landazuri, B. Bonavida, and J. L. Fahey. 1974. Cellular immunoadsorbents. An improved technique for specific depletion of cytotoxic T lymphocytes. *J. Immunol. Methods* **5**:97.
 17. Stulting, R. D., and G. Berke. 1973. Nature of lymphocyte-tumor interaction. A general method for cellular immunoadsorption. *J. Exp. Med.* **137**:932.
 18. Herberman, R. B., S. Bertram, J. S. Haskill, M. Nunn, H. T. Holden, and W. H. West. 1977. Fc receptors on mouse effector cells mediating natural cytotoxicity against tumor cells. *J. Immunol.* **119**:322.
 19. Cudkowicz, G., and E. Lotzova. 1973. Hemopoietic cell-defined components of the major histocompatibility complex of mice. Identification of responsive and unresponsive recipients to bone marrow transplants. *Transplant. Proc.* **5**:1399.
 20. Bennett, M., P. H. Pinkerton, G. Cudkowicz, and R. M. Bannerman. 1968. Hemopoietic progenitor cells in marrow and spleen of mice with hereditary iron deficiency anemia. *Blood.* **32**:908.
 21. Welsh, R. M. 1978. Cytotoxic cells induced during lymphocyte choriomeningitis virus infection in mice. I. Characterisation of natural killer cell induction. *J. Exp. Med.* **148**:163.
 22. Kumar, V., M. Bennett, and R. J. Eckner. 1974. Mechanisms of genetic resistance to Friend leukemia virus in mice. I. Role of ⁸⁹Sr-sensitive effector cells responsible for rejection of bone marrow allografts. *J. Exp. Med.* **139**:1093.
 23. Bennett, M., and E. E. Baker. 1977. Marrow dependent (M) cell function in early stages of infection with *Listeria monocytogenes*. *Cell. Immunol.* **33**:203.
 24. Lopez, C. 1978. Genetic resistance to HSV-1 in the mouse is mediated by the "M" cells of allogeneic resistance. *Fed. Proc.* **37**:1560.
 25. Merluzzi, V. J., E. M. Levy, V. Kumar, M. Bennett, and S. R. Cooperband. 1978. In vitro activation of suppressor cells from spleens of mice treated with radioactive strontium. *J. Immunol.* **121**:505.

26. Bennett, M., E. E. Baker, J. W. Eastcott, V. Kumar, and D. Yonkosky. 1976. Selective elimination of marrow precursors with the bone seeking isotope ^{89}Sr : implications for hemopoiesis, lymphopoiesis, viral leukemogenesis, and infection. *J. Reticuloendothel. Soc.* **20**: 71.
27. Cudkowicz, G. 1965. The immunogenetic basis of hybrid resistance to parental marrow grafts. In *Isoantigens and Cell interactions*. J. Palm, editor. The Wistar Institute Press, Philadelphia. 37.
28. Campbell, P. A., B. L. Martens, H. R. Cooper, and J. K. McClatchy. 1974. Requirements for bone marrow derived cells in resistance to *Listeria*. *J. Immunol.* **112**:1407.
29. Herberman, R. B., M. E. Nunn, and H. T. Holden. 1978. Low density of Thy 1 antigen on mouse effector cells mediating natural cytotoxicity against tumor cells. *J. Immunol.* **121**:304.
30. Kumar, V., L. Goldschmidt, J. W. Eastcott, and M. Bennett. 1978. Mechanisms of genetic resistance to Friend virus leukemia in mice. IV. Identification of a gene (*Fv-3*) regulating immunosuppression in vitro, and its distinction from *Fv-2* and genes regulating marrow allograft reactivity. *J. Exp. Med.* **147**:422.
31. Lindahl, K. F., A. B. Peck, and F. H. Bach. 1975. Specificity of cell-mediated lympholysis for public and private H-2 determinants. *Scand. J. Immunol.* **4**:541.
32. Martin, W. J. 1975. Immune surveillance directed against derepressed cellular and viral antigens. *Cell. Immunol.* **15**:1.
33. Roder, J. C., R. Kiessling, P. Biberfeld, and B. Andersson. 1978. Target effector interaction in the natural killer (NK) cell system. II. Isolation of NK cells and studies on the mechanism of killing. *J. Immunol.* **121**:2509.