

EXPRESSION OF MURINE *Lm-1* LOCUS
Lm-1 Determinants on Lymphocytes and Macrophages, and Effects of
Lm-1 Incompatibility on Bone Marrow Grafts

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The *Lm-1* locus controls the expression of one or more cell surface alloantigens recognized by cytotoxic T lymphocytes (CTL).¹ Earlier studies showed *Lm-1* is *Igh*-linked and located five map units telomeric to *Pre-1* on chromosome 12 (1). Two *Lm-1* alleles (*Lm-1^a* and *Lm-1^b*) were identified, and lipopolysaccharide (LPS) blasts from 46 strains derived from crosses between *Lm-1^a* and *Lm-1^b* mice were typed. 37 of these strains were known to have genetic recombinations in the telomeric region of chromosome 12. None of the recombinations divided the *Lm-1* locus; i.e., all strains typed unambiguously as either *Lm-1^a* or *Lm-1^b*. Accordingly, if *Lm-1* were to consist of more than one gene, such genes would have to be closely linked.

Two other laboratories (2, 3) have identified *Igh*-linked loci for cell surface alloantigens recognized by CTL. Forman et al. (2) have defined a locus telomeric to *Igh* and *Tsu* that codes for an antigen (H-40) that is expressed only on surface Ig-positive B cells. The antigen described by Rolink et al. (3), H(C_H), is also encoded by a locus telomeric to *Igh*, but is expressed on both B and T lymphocytes.

All of the above findings clearly demonstrate that the telomeric end of mouse chromosome 12 codes for cell surface determinants that are immunogenic for T cells. What is not clear, however, is the number of such antigens, their distribution and relationship to each other, and their possible role in cell differentiation and interaction.

To elucidate further the nature of *Lm-1* and its product(s), we tested different cell types for expression of Lm-1 determinants. We show here that Lm-1 determinants (*a*) are expressed on T and B cell blasts, some pre-B cell lines, and macrophages, but not on fibroblasts, normal (unstimulated) thymocytes, and cloned lines of two thymic T cell lymphomas; (*b*) are differentially expressed on

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¹ *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; Con A SN, supernatant from concanavalin A-activated spleen cells; CTL, cytotoxic T lymphocyte; E:T, effector/target; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HBSS, Hank's balanced salt solution; LPS, lipopolysaccharide; OD, optical density; SL, specific lysis; T', radiolabeled target cells.

T, B and pre-B cells; and (c) may serve as important histocompatibility antigens for bone marrow transplantation.

Materials and Methods

Mouse Strains

BALB/cAnNlcr and C57BL/6Nlcr are maintained in the Laboratory Animal Facility at the Institute for Cancer Research, Fox Chase Cancer Center. The *Igh^b* congenic mouse strains C.B-17, C.B-26, C.B-31, and C.B-39 have been described in detail previously (1). The AXC-11, CB-AL/1, and CXAXCB-2 strains were a gift from Dr. R. Riblet, Fox Chase Cancer Center, and were typed in the initial study (1).

Cell Lines

Abelson murine leukemia virus (A-MuLV)-induced pre-B cell lines. A-MuLV-induced lines were obtained from R. Phillips (Ontario Cancer Institute, Toronto, Canada). Those derived from in vitro transformation of C.B-17 bone marrow include C.BAbl-7, C.BAbl-11, C.BAbl-15, C.BAbl-17, and C.BAbl-7853. The lines, SAbl-11, SAbl-12, and SAbl-3 were derived from bone marrow of C.B-17^{scid} mice (11). All cell lines show rearrangements at both *Igh* alleles (I. J. Weiler, M. J. Bosma, R. Phillips, and R. Perry, unpublished results), and, where tested, react with monoclonal antibodies (12, 13) specific for pre-B and B cell determinants, but lack surface Ig (our unpublished results).

T cell lines. SCID I-12 2H4 and SCID 56 3B5 are cloned cell lines derived from T cell lymphomas (Thy-1.2⁺, Lyt-1⁺, Lyt-2⁺) that arose in C.B-17^{scid} mice (11). Both clones show gene rearrangement at the β_2 locus for the T cell antigen receptor, as revealed by Southern blot analysis using a β_2 probe from T. Mak, Ontario Cancer Institute, Toronto (I. J. Weiler, personal communication).

Immunizations

CTL used in this study were generated by immunizing mice with spleen cells from their *Lm-1*-congenic partner strains. C.B-17 and C.B-26 express *Lm-1^b*, while C.B-31 and C.B-39 express *Lm-1^a*. We have previously (1) shown that C.B-17 and C.B-26 differ from C.B-31 and C.B-39 at a segment of chromosome 12 that includes the *Pre-1* and *Lm-1* loci, but not the centromeric *Igh* or the telomeric *Ly7* loci. We refer to CTL generated in C.B-17 or C.B-26 mice against C.B-31 or C.B-39 spleen cells as anti-*Lm-1^a* effectors, and CTL generated in C.B-31 or C.B-39 mice against C.B-17 or C.B-26 spleen cells as anti-*Lm-1^b* effectors.

Spleen cells were suspended in Hank's balanced salt solution (HBSS), emulsified in complete Freund's adjuvant (CFA), and injected intraperitoneally (1/5 spleen per mouse) into congenic mice. 2 wk later, immunized mice were boosted with the same dose of spleen cells in HBSS (without CFA), rested for 2 mo, then boosted again with spleen cells in HBSS at monthly intervals. There was a 2-wk period between the last immunization and sacrifice. We did not detect anti-*Lm-1* antibodies in any mice, even after prolonged immunization.

Generation of Cytotoxic Effector Cells

Following procedures described previously (4), single-cell suspensions were prepared from spleens of immunized mice (for anti-*Lm-1* effectors) or normal C57BL/6 mice (for anti-H-2^d effectors), and from spleens of the immunizing strain (stimulator cells). Erythrocytes were lysed by treatment with NH₄Cl (5). Effector cells (at 5×10^6 cells/ml) were mixed with equal volumes of cesium-irradiated stimulator cells (2,000 rad), also at 5×10^6 cells/ml, in Costar 3075 tissue culture flasks (16 ml/flask), and cultured for 6 d in a 37°C humidified incubator. The culture medium consisted of RPMI 1640 (Microbiological Associates, Walkersville, MD), 10% fetal calf serum (Microbiological Associates), 5×10^{-5} M 2-mercaptoethanol, 50 μ g/ml gentamycin (Schering Corp., Union, NJ), 10 mM Hepes, pH 7.2, (Gibco, Grand Island, NY), and 2 mM glutamine.

Preparation of Target Cells and Splenic LPS Blasts

Cell suspensions of normal spleen cells (erythrocytes lysed by NH_4Cl treatment) were cultured at 5×10^6 cells/ml $10 \mu\text{g/ml}$ with *S. typhosa* LPS, W (Difco Laboratories, Inc., Detroit, MI) for 3 d before labeling with ^{51}Cr . Where T cell depletion is indicated, the adherent fraction from a nylon wool column (6), was recovered by adding cold medium and compressing the column with a syringe plunger.

Splenic T Cell Blasts

Spleen cells were fractionated by nylon wool filtration according to the method of Julius et al. (6). The effluent fraction was cultured at 5×10^6 cells/ml with $5 \mu\text{g/ml}$ Con A (E. Y. Laboratories, San Mateo, CA) in Costar 3075 tissue culture flasks for 2–3 d at 37°C before use as targets.

Thymocyte Blasts

Thymocytes were cultured at 5×10^6 cell/ml in medium containing 50% supernatant of concanavalin A (Con A)-stimulated spleen cells (Con A SN) at 37°C for 3 d before ^{51}Cr labeling as described above. Con A SN was prepared by harvesting the supernatant of Con A activated mouse spleen cells after 48 h in culture as described previously (7).

Fibroblast Targets

Fibroblast cultures were derived from explants from the subcutaneous connective tissue of 3-mo-old BALB/c and C.B-17 mice. Explants were originally cultured in 60-mm petri dishes (Falcon Labware, Oxnard, CA) under drops of RPMI media supplemented as described above. After 24 h, media (3 ml) was added to dishes, and the cultures were fed every 4 d. After 2 wk, fibroblasts were harvested by incubation with trypsin EDTA solution (Gibco, Grand Island, NY) and passed into Costar 3075 culture flasks. Cells were passaged when grown to monolayers, and used as targets from 1 to 1.5 mo after culture initiation.

Macrophage Targets

Mice received an intraperitoneal injection of 2 ml 3% thioglycollate medium (Difco Laboratories) 4 d before sacrifice. Peritoneal cells were harvested by lavage and plated in flat-bottom 96-well culture plates (Costar 3596) at 10^5 cells/200 $\mu\text{l/well}$. Cells were incubated overnight and washed once before use as targets.

Measurement of Macrophage Death

Following the methods of Parish and Mullbacher (9), macrophage monolayers were overlaid with the various concentrations of effector cells. After a 6-h incubation, the plates were washed once with HBSS. The number of live macrophages remaining adherent was quantitated by measuring the uptake of neutral red. To each well, 200 μl of 0.036% neutral red (Polysciences, Inc., Warrington, PA) was added. Plates were placed at 37°C for 15 min. The neutral red solution was decanted, and the wells were washed by submerging the plates three times in phosphate-buffered saline, and inverting the plates on paper towels between each wash. The macrophages were then solubilized by the addition of 200 μl of 0.5% sodium dodecyl sulfate in 0.05 M acetic acid to each well. The neutral red released from the macrophages was quantitated by measurement of optical density (OD) by a micro-ELISA (enzyme-linked immunosorbent assay) autoreader MR580 (Dynatech Laboratories, Alexandria, VA) with test wavelength 540 nm and reference wavelength 630 nm. Percent specific lysis (SL) was calculated as $100 \times (\text{control} - \text{test}) / \text{control}$, where control = OD with no effector cells present, and test = OD with effector cells present.

In this assay, both lysis and detachment of adherent macrophages caused by antigen-specific effector cells were scored as cell death. While detached macrophages are functionally compromised, they may not yet be lysed. However, as the amount of neutral red uptake was strictly correlated with the presence (or absence) of the appropriate antigen-

specific effector cells, we consider this assay a valid and reliable way to detect Lm-1 determinants on macrophages. The results were recorded as percent SL.

⁵¹Cr Labelling of Targets

Cells were suspended in 100 μ l of fetal calf serum (FCS) and 100 μ Ci Na₂⁵¹CrO₄ (New England Nuclear, Boston, MA), and incubated at 37°C for 1 h. The cells were then washed three times to remove free isotope. Targets were suspended at 10⁵ cell/ml in RPMI 1640 with 20% FCS, and 100 μ l/well were assayed for lysis in the cytotoxicity assay.

⁵¹Cr-release Assay

After 6 d in culture, the effector cells were harvested and tested in a standard ⁵¹Cr-release assay (8). All results are reported as percent SL, which is calculated as $100 \times (\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})$, where experimental release is cpm ⁵¹Cr released in the presence of effector cells. Maximum release is determined by detergent lysis. All values reported represent the mean of quadruplicate measurements. In all cases, the SE was <5% and is, therefore, not given.

For each experiment shown, four fivefold dilutions of effectors were tested against a fixed number of targets. The starting effector/target ratio (E:T) was between 100:1 and 50:1. Except for cold-target inhibition experiments, (see below) data are reported as percent SL at the highest E:T ratio only. This ratio was chosen to demonstrate that, even at the highest E:T ratio, the targets that we have designated as negative for lysis are truly negative. However, when a given target is susceptible to lysis, the percent SL at the lowest E:T ratio was usually at least 50% that seen at the highest E:T ratio. The E:T ratio used to obtain the given values are noted in the table.

Cold-target Inhibition of Anti-Lm-1 Effector Cells

Effector cell lysis of labeled target cells was inhibited by addition of unlabeled target cells. Effector cell cultures were sampled on the fifth day, and the cells were titrated against a fixed number of labeled target cells to determine limiting effector/labeled target cell ratios (E:T'); i.e., the smallest ratio still to give significant lysis of labeled targets. In different experiments, limiting E:T' ratios ranged from 5:1 to 0.15:1, and they are noted in the legends. On the sixth day of culture, effector cells were harvested for cold-target inhibition assays (14). Varying numbers of unlabeled target cells were added at limiting E:T' ratios. The ratio of unlabeled/labeled target cells (T:T') is indicated in the figures.

The procedure for each assay was as follows. Effector cells (100 μ l) were added to microtiter wells, followed by unlabeled target cells (50 μ l). Labeled target cells (also in 50 μ l) were added last. The cells were gently pelleted by centrifugation for 4 min at 300 rpm, and incubated for 3–4 h. Percent inhibition of lysis by unlabeled target cells is calculated as $100 \times [1 - (\text{percent SL with added T}) / (\text{percent SL with no added T})]$. Percent SL is given as the mean of quadruplicate determinations for each indicated T:T' ratio. In all experiments shown, the SE was <3%, and therefore is not given.

Fluorescence-activated Cell Sorter (FACS) Analysis

Cells were labeled with an optimal amount of fluorescein isothiocyanate (FITC)-conjugated monoclonal rat anti-Thy-1.2 antibody (10), or FITC-conjugated goat anti-mouse (Fab)₂ sera (Cappel Laboratories, Cochranville, PA). All unreacted antibody was removed by washing, and the cells were analyzed with a FACS II system (Becton Dickinson and Company, Sunnyvale, CA), using 488 nm laser illumination.

In Vivo Cell Transfers

One day before cell transfer, recipient mice received 600 rad irradiation from a X-ray source under the following conditions: 225 kV; filtration 1 mm Al and 0.5 mm Cu; dose rate 35–40 rad/min. Mice were injected intravenously with 0.5 ml HBSS containing 10⁷ spleen cells and 2×10^6 bone marrow cells. At various times thereafter, they were bled, and the sera were tested for Igh allotype markers.

Antiallotype Assay

The expression of Igh allotypes was assayed by means of immunoprecipitation in Ouchterlony microplates. The preparation of these antisera and their specificity has been described previously (15).

Results

Expression of Lm-1 Determinants on Splenic T Cell Blasts and Activated Thymocytes. ^{51}Cr targets were prepared from splenic T cell blasts, fresh thymocytes, and thymocytes after 1, 2, and 3 d of in vitro culture with Con A SN. Splenic B cell blasts were included as controls. Targets were tested for susceptibility to lysis by both anti-Lm-1 effectors and anti-H-2^d effectors. All targets were lysed by anti-H-2^d effectors (Table I). Anti-Lm-1^a effectors killed both splenic T and B cell blasts from the *Lm-1^a* strain, C.B-31 (Table I). Target cells from the *Lm-1^b* strain, C.B-26, were unaffected. In the reciprocal experiment, anti-Lm-1^b effectors killed splenic T and B cell blasts from C.B-26 but not from C.B-31. Fresh thymocytes and thymocytes after 1 d in culture with Con A SN were not susceptible to lysis by anti-Lm-1 effectors. When cultured for 2–3 d with Con A SN, however, thymocytes become susceptible to lysis (Table I). Activation with Con A SN, therefore, results in the conversion of thymocytes from Lm-1⁻ to Lm-1⁺.

Two Independent Cultured Lines of Thymic T Cell Lymphomas Do Not Express Lm-1 Determinants. The cloned T cell lymphomas, SCID 56 3B5 and SCID I-12

TABLE I
Expression of Lm-1 Determinants on Thymic and Splenic T Cell Blasts

Target strain	Target cell type	Target activator	Time in culture with activator	SL with various effectors:		
				Anti-Lm-1 ^b	Anti-Lm-1 ^a	Anti-H-2 ^d
			<i>d</i>		<i>%</i>	
C.B-26	Splenic B cells*	LPS	3	62	1	75
C.B-26	Splenic T cells [‡]	Con A	3	39	0	72
C.B-26	Thymocytes	Con A SN	0	0	0	40
C.B-26	Thymocytes	Con A SN	1	0	0	45
C.B-26	Thymocytes	Con A SN	2	25	0	65
C.B-26	Thymocytes	Con A SN	3	57	0	60
C.B-31	Splenic B cells	LPS	3	0	79	81
C.B-31	Splenic T cells [‡]	Con A	3	0	41	69
C.B-31	Thymocytes	Con A SN	0	0	0	51
C.B-31	Thymocytes	Con A SN	1	0	42	60
C.B-31	Thymocytes	Con A SN	3	0	42	60

All values obtained with E:T = 100:1.

* B cells were prepared by culturing the nylon wool-adherent fraction of spleen cells with LPS for 3 d before use as targets. These cells were 92% surface Ig-positive by FACS analysis.

[‡] T cells were prepared from the nylon wool-effluent fraction of a spleen cell suspension, and cultured with Con A for 3 d before use as targets. These cells were 95% Thy-1.2⁺ by FACS analysis.

TABLE II
Thymic T Cell Lymphomas of C.B-17scid Do Not Express Lm-1 Determinants

In vitro stimulator	Target source	Target cell type	SL with effectors:	
			Anti-Lm-1 ^b *	Anti-H-2 ^d †
			%	
C.B-17	C.B-17	LPS blasts	42	62
C.B-17	BALB/c	LPS blasts	0	59
C.B-17	C.B-17 ^{scid}	SCID-56 3B5	2	34
C.B-17	C.B-17 ^{scid}	SCID I-12 2H4	0	36
SCID I-12 2H4	C.B-17	LPS blasts	0	64
SCID 56 3B5	C.B-17	LPS blasts	0	62

* E:T = 50:1.

† E:T = 25:1.

2H4, were both tested for Lm-1 expression. Neither cell line could stimulate, nor be lysed by, anti-Lm-1^b effectors (Table II). Controls show that both T cell clones could stimulate and be lysed by anti-H-2^d effectors. We consider these clones (Thy-1.2⁺, Lyt-1⁺, Lyt-2⁺) examples of immature T cells, as they were derived from two independent thymic T cell lymphomas of C.B-17^{scid} mice. These mice lack functional T cells (11).

Variable Expression of Lm-1 Determinants on Pre-B Cell Lines Induced by A-MuLV. A number of A-MuLV-induced cell lines of early B cell phenotype were tested for susceptibility to lysis by anti-Lm-1 effectors. With respect to cell lines of C.B-17 origin, C.BAbl-7853, C.BAbl-11, and C.BAbl-15 expressed Lm-1 determinants, and lines C.BAbl-17 and C.BAbl-7 did not. Of the cell lines of C.B-17^{scid} origin, SAbl-11 and SAbl-12 were Lm-1⁺, and SAbl-3 was Lm-1⁻ (Table III). All targets were susceptible to lysis by anti-H-2^d effectors.

Expression of Lm-1 Determinants on Macrophages but Not on Fibroblasts. Anti-Lm-1^a and anti-Lm-1^b effectors were tested for the ability to kill macrophages from both *Lm-1^a* and *Lm-1^b* strains. Anti-Lm-1^a effectors killed macrophages from the *Lm-1^a* strains, BALB/c, C.B-39, and C.B-31 but not from the *Lm-1^b* strains, C.B-17 and C.B-26 (Table IV). Conversely, anti-Lm-1^b effectors lysed macrophages from C.B-17, C.B-26 (Table IV), and C.B-17^{scid} mice (data not shown). The results shown in Table IV on Lm-1 expression by macrophages were obtained using the neutral red assay described in Materials and Methods. This assay was chosen to demonstrate clearly the phagocytic ability of the target cell. However, these results have been repeated using a conventional ⁵¹Cr release assay with labeled, thioglycollate-induced, peritoneal adherent cells as targets (data not shown).

Fibroblast lines derived from the *Lm-1^a* and *Lm-1^b* strains were also tested for susceptibility to lysis. As shown in Table IV, these lines were lysed by anti-H-2^d effector cells but not by anti-Lm-1 effectors cells. Not shown is that fibroblasts were unable to substitute for spleen cells as in vitro stimulators of anti-Lm-1 effectors. Further evidence that fibroblasts do not express Lm-1 determinants

TABLE III
Variable Expression of Lm-1 Determinants on A-MuLV-induced B Cell Lines

Target strain origin	Cell type	SL with effectors:		
		Anti-Lm-1 ^{b*}	Anti-Lm-1 ^{a*}	Anti-H-2 ^{d†}
			%	
C.B-31	LPS blasts	1	47	47
C.B-26	LPS blasts	62	0	58
C.B-17	C.BAbl-7853	42	0	58
C.B-17	C.BAbl-11	13	0	Not done
C.B-17	C.BAbl-15	11	0	40
C.B-17	C.BAbl-17	0	0	60
C.B-17	C.BAbl-7	0	0	45
C.B-17 ^{scid}	SAbl-11	33	0	45
C.B-17 ^{scid}	SAbl-12	32	0	27
C.B-17 ^{scid}	SAbl-3	0	0	57

* E:T = 80:1.

† E:T = 100:1.

TABLE IV
Expression of Lm-1 Determinants on Macrophages but Not on Fibroblasts

Target strain	Target cell type	SL with effectors:		
		Anti-Lm-1 ^b	Anti-Lm-1 ^a	Anti-H-2 ^d
			%	
C.B-17	Macrophages	47	0	48
C.B-17	Fibroblasts	4	0	91
	Splenic LPS blasts	75	0	89
C.B-26	Macrophages	38	0	75
	Splenic LPS blasts	52	0	74
BALB/c	Macrophages	0	54	70
	Fibroblasts	0	0	80
	Splenic LPS blasts	0	62	76
C.B-31	Macrophages	0	49	56
	Splenic LPS blasts	0	62	85
C.B-39	Macrophages	0	48	60
	Splenic LPS blasts	0	69	88

All values obtained with E:T = 100:1.

comes from cold-target inhibition studies. BALB/c fibroblasts did not inhibit the killing of BALB/c LPS blasts by anti-Lm-1^a effectors, but did inhibit killing by anti-H-2^d effectors (data not shown).

A Common Lm-1 Determinant on T and B Cells that Is Not Expressed on Pre-B Cells. The ability of thymocyte blasts and splenic B cell blasts to inhibit the lysis of labeled thymocyte blasts is shown in Fig. 1. Both sources of unlabeled blasts inhibited anti-Lm-1^b (Fig. 1A) and anti-Lm-1^a (Fig. 1B) effector cell lysis by ~80% at T:T' ratios $\geq 10:1$. This inhibition was Lm-1 specific, since unlabeled blasts of the alternative Lm-1 allele did not inhibit lysis. Results similar to those of Fig. 1 were also obtained when purified populations of unlabeled splenic T and B cells were used to inhibit lysis of splenic T cell blasts (data not shown).

In contrast to B cell blasts, the Lm-1⁺ pre-B cell lines, SAb1-11 and C.BAb1-7853, failed to inhibit effector cell lysis of thymocyte blasts even at T:T' ratios of 20:1 (data shown for SAb1-11 only) (Fig. 1B). We conclude there is an Lm-1 determinant common to both T and B cells that is apparently not expressed on Lm-1^b-positive pre-B cells. We tentatively designate this determinant Lm-1.1 for Lm-1^a strains and Lm-1.11 for Lm-1^b strains.

A Common Lm-1 Determinant on Pre-B and B Cells that Is Not Expressed on T Cells. Cold-target inhibition experiments were also performed using the Lm-1^b pre-B cell lines, SAb1-11 and C.BAb1-7853 as the labeled target cells. As shown in Fig. 2, B cell blasts were more effective inhibitors of pre-B cell lysis than the pre-B cells themselves. This difference is presumably due to a higher density of Lm-1 determinants on B cells relative to pre-B cells. Nonetheless, both sources of unlabeled targets inhibited lysis (percent inhibition $\geq 70\%$ at T:T' ratios of 10:1). Thymocyte blasts, on the other hand, were ineffective inhibitors of pre-B cell lysis. Maximal inhibition in this case was $<30\%$. These data indicate that

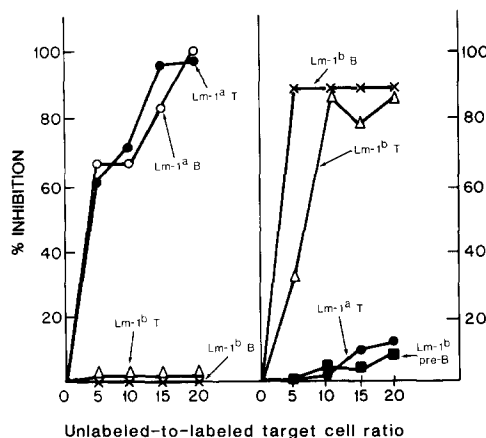


FIGURE 1. Ability of various unlabeled target cells to inhibit effector cell lysis of labeled T cells. (A) C.B-26 anti-C.B-31 effector cell lysis of Lm-1^a-labeled T cells (where E:T' = 2:1 and percent SL = 19) is inhibited by Lm-1^a T and Lm-1^a B cells, but not by Lm-1^b T and Lm-1^b B cells. (B) C.B-31 anti-C.B-26 effector cell lysis of Lm-1^b cells (where E:T' = 1:1 and percent SL = 20) is inhibited by Lm-1^b T and Lm-1^b B cells, but not by Lm-1^a T cells and Lm-1^b pre-B cells (SAb1-11). Lm-1^a and Lm-1^b T cells were C.B-31 and C.B-26 Con A SN-activated thymocytes; Lm-1^a and Lm-1^b B cells were C.B-31 and C.B-26 LPS-activated spleen cells depleted of T cells by passage over nylon wool before activation.

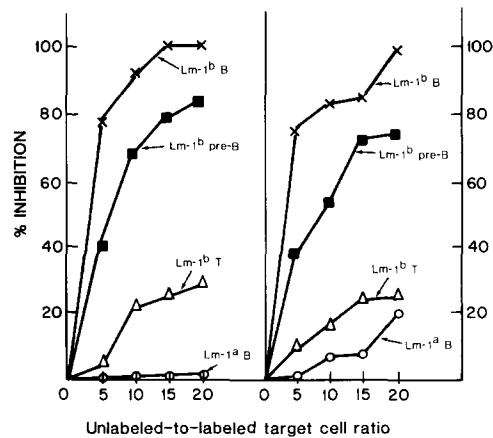


FIGURE 2. Ability of various unlabeled target cells to inhibit effector cell lysis of labeled *Lm-1^b* pre-B cell lines. (A) and (B) C.B-31 anti-C.B-26 effector cell lysis of labeled SAb1-11 and C.BAb1-7853 pre-B cell lines is inhibited by SAb1-11 (A), C.BAb1-7853 (B), and *Lm-1^b* B cells, but only partially inhibited by *Lm-1^b* T cells, and not at all by *Lm-1^a* B cells. *Lm-1^b* T cells were C.B-26 Con A SN-activated thymocytes; *Lm-1^a* and *Lm-1^b* B cells were C.B-31- and C.B-26-activated spleen cells depleted of T cells by passage over nylon wool before activation. Conditions: E:T' = 3:1 and percent SL = 17 in A; E:T' = 5:1 and percent SL = 20 in B.

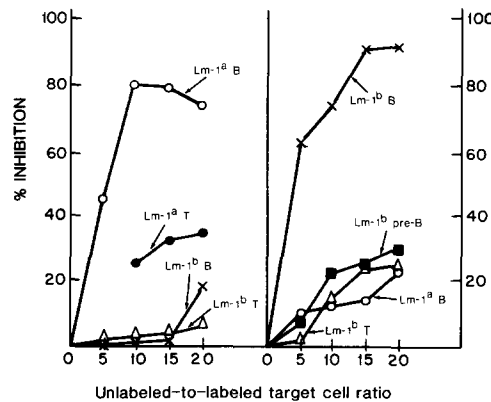


FIGURE 3. Ability of various unlabeled targets to inhibit effector cell lysis of labeled B cells. (A) C.B-26 anti-C.B-31 effector cell lysis of *Lm-1^a*-labeled B cells (where E:T' = 0.3:1 and percent SL = 35) is inhibited by *Lm-1^a* B cells, but only partially inhibited by *Lm-1^a* T cells, and not at all by *Lm-1^b* T or B cells. (B) C.B-31 anti-C.B-26 effector cell lysis of *Lm-1^b*-labeled B cells (where E:T' = 0.2:1 and percent SL = 40) is inhibited by *Lm-1^b* B cells, but not appreciably by *Lm-1^b* T. *Lm-1^b* pre-B cells were the C.BAb1-7853 cell line. *Lm-1^a* and *Lm-1^b* T cells were C.B-31 and C.B-26 Con A SN-activated thymocytes; *Lm-1^a* and *Lm-1^b* B cells were C.B-31 and C.B-26 LPS-activated spleen cells depleted of T cells by passage over nylon wool before activation.

there is another *Lm-1* determinant, common to both pre-B and B cells, but absent from T cells, or poorly expressed on such cells. This determinant will be designated *Lm-1.2* for *Lm-1^a* strains and *Lm-1.12* for *Lm-1^b* strains.

Inability of Pre-B and T Cells to Inhibit Lysis of B Cells. Reciprocal experiments to those of Figs. 1 and 2 are shown in Fig. 3. Here, we compare the ability of pre-B cells, B cell blasts, and thymocyte blasts to inhibit anti-*Lm-1* effector cell-

mediated lysis of labeled B cell blasts. The inhibition observed with pre-B cell inhibitors was always negligible, and that observed with thymocytes was usually $\leq 15\%$ at T:T' of 20:1. Purified populations of splenic T cell blasts and SABL-11 pre-B cell line also failed to inhibit lysis (data not shown). Occasionally, some inhibition (20–30%) was observed when *Lm-1^a* T cells were tested as inhibitors (Fig. 3A). The reason for this is not clear, but it might be explained by contamination of the B cell target preparations with T cells.

Our interpretation of the results in Fig. 3B in light of Figs. 1 and 2 is as follows: *Lm-1^b* B cells express at least two determinants (Lm-1.11 and Lm-1.12), one of which is on *Lm-1^b* T cells (Lm-1.11), and the other on *Lm-1^b* pre-B cells (Lm-1.12). Therefore, the addition of *Lm-1^b* T or *Lm-1^b* pre-B cells alone would be expected to inhibit only one of two anti-*Lm-1^b* effector cell populations, leaving the other population free to lyse B cell targets.

This interpretation helps explain the apparent differences in lytic efficiency with which anti-*Lm-1* effectors lysed B cell blasts, thymocyte blasts and the pre-B cell lines. This is indicated by the limiting E:T' ratios for each labeled target in the legends of Figs. 1, 2, and 3. As can be noted, B cell blasts were lysed ~ 10 times more efficiently than thymocyte blasts, and ~ 25 times more efficiently than pre-B cells (the mean E:T' ratio for B cell blasts was 0.15:1 vs. 1.5:1 for thymocyte blasts, and 4:1 for pre-B cells).

Effect of Mixtures of T and Pre-B Inhibitors on B Cell Lysis. The data in Fig. 3 leave open the possibility that B cells may express a third *Lm-1* determinant distinct from both *Lm-1.1* (Lm-1.11) and *Lm-1.2* (Lm-1.12). We attempted to test this by inhibiting B cell lysis with mixtures of T and pre-B cells. As shown in Table V, at T:T' ratios of 10:1 to 20:1, *Lm-1^b* T cells or pre-B cells alone gave $\leq 16\%$ inhibition (groups B and C), while *Lm-1^b* B cells gave 78–95% inhibition (group A). *Lm-1^b* pre-B cells mixed with *Lm-1^b* T cells, each at a T:T' of 10:1, gave 28% inhibition (group B). This was only marginally better than the 13% inhibition obtained with mixtures of *Lm-1^b* pre-B cells and irrelevant *Lm-1^a* T cells (group F). At higher T:T' ratios (total T:T' of 40:1), inhibition values in the admixture experiments became meaningless, as even irrelevant cells began to inhibit lysis (see group F).

The relative inability of mixtures of *Lm-1^b* pre-B and T cells to inhibit *Lm-1^b* B cell lysis suggests that a third *Lm-1* determinant may be present on B cells. This interpretation, however, must be regarded as preliminary. Given two *Lm-1^b* determinants (Lm-1.11 and Lm-1.12) on *Lm-1^b* B cells and two *Lm-1^b*-specific CTL populations, there may be cooperative effects in the lysis of *Lm-1^b* B cells. Moreover, possible differences in the density of *Lm-1^b* determinants on pre-B, B, and T cells could complicate the interpretation of Table V. Further analysis of distinct *Lm-1* determinants awaits the availability of *Lm-1*-specific CTL clones.

Genetic Linkage of Lm-1.1 and Lm-1.2 (and Lm-1.11 and Lm-1.12) Determinants. The demonstration of distinct *Lm-1* determinants suggested that separate genes might code for these determinants and that these might have segregated in strains known to be chromosomal recombinants in the region near *Lm-1*. Therefore, thymocyte and B cell blasts were prepared from ten of the recombinant strains that were most informative in the previous *Lm-1* mapping study (1). Each cell type was tested for susceptibility to lysis by both anti-*Lm-1^a*

TABLE V
Ability of T and Pre-B Cell Mixtures to Inhibit Anti-Lm-1^b Effector
Cell Lysis of Labeled Lm-1^b B Cells

Group	Inhibitor 1		Inhibitor 2		Final T:T'	Inhibition
	Cell	T:T'	Cell	T:T'		
						%
A	Lm-1 ^b	5:1			5:1	65
	B Cells	10:1			10:1	78
		20:1			20:1	95
B	Lm-1 ^b	5:1			5:1	5
	T cells*	10:1			10:1	12
		20:1			20:1	16
C	Lm-1 ^b	5:1			5:1	6
	Pre-B	10:1			10:1	10
	Cells	20:1			20:1	12
D	Lm-1 ^b	5:1	Lm-1 ^b	5:1	10:1	13
	T cells*	10:1	Pre-B	10:1	20:1	28
		20:1		20:1	40:1	65
E	Lm-1 ^a	10:1			10:1	2
	T cells*	20:1			20:1	6
		40:1			40:1	40
F	Lm-1 ^a	5:1	Lm-1 ^b	5:1	10:1	9
	T cells	10:1	Pre-B	10:1	20:1	13
		20:1		20:1	40:1	48

E:T' = 0.2:1. Percent SL was 25%.

* Con A SN-activated thymocytes.

and anti-Lm-1^b effector cells. The data shown in Table VI are representative of the results. No recombinants between the *Lm-1^a* and *Lm-1^b* alleles have, as yet, been found. Therefore, if the *Lm-1^a* and *Lm-1^b* loci were to contain separate genes for Lm-1.1/Lm-1.2 and Lm-1.11/Lm-1.12, respectively, these genes must be closely linked.

In Vivo Effect of Anti-Lm-1 T Cells on B Cells. Spleen cells from mice immune to Lm-1^a (or Lm-1^b) were mixed with bone marrow cells from *Lm-1^a* (or *Lm-1^b*) mice. The mixture was then injected intravenously into X-irradiated recipients of the same *Lm-1* phenotype as the spleen cell donors. At various time intervals, sera from the recipients were tested for the presence of allotype of the bone marrow donor. C.B-26 anti-C.B-31 spleen cells were the source of anti-Lm-1^a cells, and BALB/c anti-C.B-17^{scid} spleen cells were the source of anti-Lm-1^b cells. The possibility of an anti-Igh^b response was excluded as C.B-17^{scid} spleen cells are surface Ig-negative (11).

The results were striking, as shown in Table VII. Control recipients (groups C, D, H, I, and J) showed Igh-allotype production by B cells from the bone marrow graft within 3–13 wk after cell transfer. Whereas, with one exception, no detectable Igh allotypes of bone marrow graft origin appeared in this time period in experimental groups that received anti-Lm-1 immune cells (A and E). The inhibition was specific for the relevant *Lm-1* allele, as demonstrated in group F, where anti-Lm-1^b immune spleen cells did not prevent production of Igh^b allotype by bone marrow-derived Lm-1^a B cells. When unprimed spleen cells

TABLE VI
Lm-1 Typing of Thymocyte Blasts from Recombinant Strains

Target strain	Igh-c	Tsu ^d	Pre-1	Lm-1	Effectors	SL with:*			
						Splenic LPS blasts	Thymic Con A SN blasts		
						%			
C.B-31	b	NK [‡]	×	a	a	anti-Lm-1 ^a	85	26	
						anti-Lm-1 ^b	2	0	
C.B-26	b	NK [‡]		o	b	anti-Lm-1 ^a	4	1	
						anti-Lm-1 ^b	46	19	
AXC-11	a	×	+	o	×	a	anti-Lm-1 ^a	62	33
							anti-Lm-1 ^b	0	4
C.B-AL/1	b	—	×	a	a	anti-Lm-1 ^a	63	17	
						anti-Lm-1 ^b	4	2	
CXAXCB-2	a	—	×	o	×	a	anti-Lm-1 ^a	83	23
							anti-Lm-1 ^b	5	0

AXC-11 and CXAXCB-2 are the only two strains (out of 46 tested) that have recombinations between *Pre-1* and *Lm-1*. All recombinant and *Lm-1* mapping data is given or cited in reference 1.

* E:T = 150:1 for both types of effector cells.

[‡] NK, not known, since both parental strains are Tsu^d.

TABLE VII
Ig Production by Cells from Bone Marrow Grafts Is Inhibited by Anti-Lm-1 Immune Spleen Cells

Group	Spleen cells	Bone marrow cells	Irradiated recipients	Number of recipients showing allo-type of bone marrow grafts/number tested at weeks after cell transfer:			
				3	13	20	30
A	C.B-26 anti-C.B-31	BALB/c	C.B-17	0/15	0/15	0/15	3/10
B	C.B-26	BALB/c	C.B-17	1/15	5/15	14/14	10/10
C	C.B-31	BALB/c	C.B-17	5/10	10/10	10/10	10/10
D	None	BALB/c	C.B-17	11/15	15/15	15/15	10/10
E	BALB/c anti-C.B-17 ^{scid}	C.B-17	BALB/c	1/10	1/10		6/10
F	BALB/c anti-C.B-17 ^{scid}	C.B-31	BALB/c	9/10	10/10		10/10
G	BALB/c	C.B-17	BALB/c	1/10	10/10		10/10
H	BALB/c	C.B-31	BALB/c	6/10	8/8		8/8
I	None	C.B-17	BALB/c	9/10	10/10		10/10
J	None	C.B-31	BALB/c	9/10	10/10		10/10

Irradiated recipients were injected with 0.5 ml HBSS containing 10⁷ spleen cells and 2 × 10⁶ bone marrow cells.

were cotransferred with bone marrow cells of a different Lm-1 type, the appearance of Igh allotype from the bone marrow graft was delayed (Table VII, groups B and G compared to C and H).

7 mo after cell transfer, Igh allotype production by donor bone marrow-derived cells was observed in some recipients (Table VII, groups A and E) indicating that successful engraftment of bone marrow cells occurred in at least some (if not all) recipients. The implications of this are considered in the Discussion.

Discussion

Expression of Distinct Lm-1 Determinants on Different Cell Types. We have shown that antigenic determinants encoded by the *Lm-1* locus are expressed on B and T cell blasts, some pre-B cell lines, and macrophages. That expression of these determinants is dependent on the stage of cell differentiation or state of cell activation is inferred from the following: First, Lm-1 determinants are not detected on fibroblasts (Table IV). Lm-1 is not, therefore, a constitutive membrane protein of all cells. Also, we have found immature B and T cell lines that do not express Lm-1 determinants (Tables II and III). Unstimulated thymocytes also do not express Lm-1 determinants, whereas thymocyte blasts and splenic T cell blasts are Lm-1⁺ (Table I). These results indicate that Lm-1 is a differentiation antigen that is expressed by lymphoid cells as they mature.

We have shown that distinct Lm-1 determinants are differentially expressed on T, pre-B, and B cells. Two Lm-1 determinants were defined on the basis of cold-target inhibition assays. These were designated Lm-1.1 and Lm-1.2 for *Lm-1^a* mouse strains and Lm-1.11 and Lm-1.12 for *Lm-1^b* mouse strains. Lm-1^b-expressing T, pre-B, and B cells typed Lm-1.11⁺/Lm-1.12⁻, Lm-1.11⁻/Lm-1.12⁺, and Lm-1.11⁺/Lm-1.12⁺, respectively. Similarly, Lm-1^a-expressing T and B cells typed Lm-1.1⁺/Lm-1.2⁻ and Lm-1.1⁺/Lm-1.2⁺, respectively. Lm-1^a pre-B cell lines from the *Lm-1^a* congenic strains, BALB/cAnNlcr or C.B-31, were not available, but presumably express only Lm-1.2. Evidence for a third Lm-1 determinant on B cells was presented (Table V), but confirmation awaits the availability of Lm-1-specific T cell clones.

Whether a given determinant is detected may depend on the immunization protocol used to generate effector T cells. For example, with our protocol, which included the use of adjuvant, mice had to be immunized at least four times to obtain effector cells that lysed macrophages, whereas two immunizations were sufficient to generate effector cells that lysed T, B, and pre-B cells (data not shown). Since the increased number of immunizations did not increase the number or efficiency of CTL specific for lymphocytes, these data suggest that macrophages may express an additional *Lm-1* determinant.

The Lm-1 Locus and its Relationship to Other Igh-linked Alloantigens. The finding that different immunization protocols may result in the detection of distinct determinants suggests an explanation for reported differences on the cell distribution of *Igh*-linked alloantigens detected by CTL. As mentioned in the introduction, two other reported (2, 3) cell-surface antigens, H-40 and H(C_H), are encoded by genes on chromosome 12, telomeric to *Igh*. Like Lm-1, both are defined as a target antigen in a CTL assay. Their map position relative to *Lm-1*

and/or *Pre-1* has not yet been determined. However, given our preliminary evidence (Table V) for a third Lm-1 determinant on B cells, it is possible that H-40 (2) is a B cell-specific member of the Lm-1 family of determinants. Similarly, H(C_H) (3), which is expressed on both B and T cell blasts, may correspond to Lm-1.1. *Lm-1*, *H-40*, and *H(C_H)* may all be members of a genetic complex that codes for a family of lymphoid and macrophage differentiation antigens. Forman et al. (2) have found that H-40 expression accompanies surface Ig expression, and have suggested therefore that H-40 is important in B cell function. Given our data on the complexity of the Lm-1 antigen system, the pattern of expression of these antigens and their modulation during lymphocyte activation, it seems likely that the *Lm-1* locus is important for the function and interaction of lymphocytes and macrophages.

In addition to H-40 and H(C_H), there is another cell surface antigen encoded on the telomeric portion of chromosome 12 that could also be a member of the Lm-1 family of antigens. The gene for the chemically induced, tumor-specific transplantation antigen, Meth A, has been mapped to the telomeric portion of chromosome 12 (16), probably telomeric to *Pre-1* (see ref. 1). Biochemical analyses of different methylcholanthrene-induced, tumor-specific transplantation antigens has led DuBois et al. (17) to propose that the Meth A antigen itself is the product of a member of a multigene family. In independent studies, Flood et al. (18) reported evidence that the Meth A antigen is serologically crossreactive with T cell-derived factors. This suggests that the normal homologue of Meth A is involved in lymphocyte function. These data strengthen our hypothesis that the telomeric end of chromosome 12 codes for a family of cell surface antigens that play a role in the differentiation and function of lymphocytes and macrophages.

Lm-1 is similar in many ways to the T-200 family of antigens (19–31). Cell-specific antigenic determinants of the T-200 family have been identified for T cells, B cells, and macrophages (25–27). Thus, T-200 shares with Lm-1 the characteristics of lymphocyte-macrophage specificity (22), regulated expression during differentiation (28), and differential expression of distinct antigenic determinants (25–27). These characteristics have made the T-200 antigen system of interest to many who study hemopoiesis, lymphocyte differentiation, and the function of cell-specific membrane molecules (25–30).

Despite their similarities, Lm-1 and T-200 can be distinguished on genetic grounds. Lm-1 is coded by one or more genes on chromosome 12 (1), whereas Ly-5, an antigenic determinant common to all members of the T-200 family, is coded by a gene on chromosome 1 (23, 24, 31). Lm-1, therefore, represents a newly identified antigen system whose potential usefulness to the study of lymphoid and myeloid differentiation resembles that of T-200.

The molecular basis for distinct Lm-1 (or T-200) determinants is not understood. Different Lm-1 determinants might result from cell-specific modification of one or more Lm-1 polypeptides before insertion into the membrane. Precedent for this idea comes from a number of systems. Thy-1 molecules show differences between cell types due to posttranslational modification (32), and differences in function between Ia molecules on B cells and macrophages have been associated with carbohydrate moieties (33). Also, evidence that the same

T-200 molecule can display different antigenic determinants (26) is consistent with this idea. Each Lm-1 determinant, on the other hand, could represent a separate gene product in the form of a single polypeptide or heteropolymer on the cell surface. Such *Lm-1* genes would have to be closely-linked (Table VI), and presumably would be structurally and functionally related.

Effects of Lm-1-incompatibility on B Lymphocyte Function. Cell transfer experiments showed that anti-Lm-1 T cells (presumably CTL) can prevent Igh allotype production in vivo. Injection of X-irradiated *Lm-1^b* recipients with bone marrow cells from *Lm-1^a* mice together with anti-Lm-1^a-specific spleen cells resulted in inhibition of Igh^a allotype production of the bone marrow-derived donor B cells. Similar results were obtained in the reciprocal experiment. The bone marrow grafts were apparently not rejected, as is evident from the appearance of Igh^a (or Igh^b) allotype in some recipients 7 mo after cell transfer. To explain these results we reason as follows: X-irradiated recipients were engrafted with Lm-1⁻ stem cells from the bone marrow graft (that such cells exist is inferred from our detection of Lm-1⁻ A-MuLV pre-B cell lines). As progeny of these stem cells matured and expressed Lm-1 determinants, they were killed (or suppressed) by anti-Lm-1 effector cells. However, with the presumed death of donor anti-Lm-1 effector cells 5–7 mo after cell transfer, some bone marrow stem cells were able to differentiate into Lm-1⁺, Igh-producing B cells. Their ability to do so may have depended on whether the host had become tolerant or immune to cells expressing the foreign Lm-1 determinants.

Although we did not test the above recipients for Lm-1-expressing macrophages and T cells of donor bone marrow origin, it seems likely that differentiation and maturation of these cell types may also have been prevented or inhibited.

Finally, the implications of Lm-1-incompatibility are worth noting. Incompatibility at the *Lm-1* locus could help explain the previously observed *Igh*-linked restriction in T-B and T-T cell-cell interactions (34–40). For example, reports that the induction (34, 35) or activity (37) of T cell-suppressor factors is restricted by *Igh*-linked genes may in fact reflect a restriction imposed by *Lm-1* or other closely-linked loci. Further, the lack of synergy observed by L'Age-Stehr (38) between T and B cells of C.B-17 and BALB/c mice may have been due to anti-Lm-1-specific T cell aggression against B cells. Second, relevant to bone marrow transplantation in general, incompatibility at *Lm-1* and its human counterpart could contribute to graft-vs.-host disease, and/or could severely impair the growth and differentiation of engrafted stem cells.

Summary

Lm-1 is an *Igh*-linked locus that codes for cell surface alloantigens (Lm-1 determinants) recognized by T lymphocytes. Using *Lm-1* congenic strains and cold-target inhibition of anti-Lm-1-specific lysis by cytotoxic T lymphocytes, we were able to demonstrate differential expression of two distinct Lm-1 antigenic determinants. One determinant is expressed on the surface of T cell blasts, the other on a number of pre-B cell lines. Both determinants are present on B cell blasts. Macrophages also bear Lm-1 determinants, and possibly express a determinant not found on lymphocytes. Fibroblasts, (unstimulated) thymocytes, and immature T cells lack detectable Lm-1 determinants. These data indicate that

expression of the *Lm-1* locus is dependent on cell lineage and the stage of cell differentiation or activation. We propose that *Lm-1* is a lymphocyte-macrophage differentiation locus containing a number of structurally and functionally related genes.

Evidence was presented that *Lm-1* may also serve as a histocompatibility locus of major importance for bone marrow transplantation. Specifically, when *Lm-1*-incompatible bone marrow cells and spleen cells (from normal or anti-*Lm-1* immune mice) were transplanted into X-irradiated recipients, the maturation and/or function of bone marrow-derived donor B cells was delayed or inhibited.

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References

1. O'Toole, M. M., R. Riblet, and M. J. Bosma. 1984. Identification and mapping of *Lm-1*, an *Igh* linked locus for a murine alloantigen. *Immunogenetics*. 20:265.
2. Forman, J., R. Riblet, K. Brooks, E. S. Vittetta, and L. A. Henderson. 1984. H-40, an antigen controlled by an *Igh* linked gene and recognized by cytotoxic T lymphocytes. I. Genetic analysis of H-40 and distribution of its products on B cell tumors. *J. Exp. Med.* 159:1724.
3. Rolink, T., K. Eichmann, and M. M. Simon. 1978. Detection of two allotype-(Ig-1)-linked minor histocompatibility loci by use of H-2-restricted cytotoxic lymphocytes in congenic mice. *Immunogenetics*. 7:321.
4. Grabstein, K., and Y. U. Chen. 1980. Cell mediated cytolytic responses. In *Selected Methods in Cellular Immunology*. B. B. Mishel and S. M. Shiigi, editors. W. H. Freeman and Co., San Francisco. 124-137.
5. Boyle, W. 1968. An extension of the ⁵¹Cr-release assay for the estimation of mouse cytotoxins. *Transplantation (Baltimore)*. 6:761.
6. Julius, M., E. Simpson, and L. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
7. Gillis, S., M. M. Fern, W. Ou, and K. A. Smith. 1978. T cell growth factor: Parameters for production and a quantitative microassay for activity. *J. Immunol.* 120:2027.
8. Thorn, R. M., J. C. Palmer, and L. A. Manson. 1974. A simplified ⁵¹Cr-release assay for killer cells. *J. Immunol. Methods*. 4:301.
9. Parish, C. R., and A. Mullbacher. 1983. Automated colormetric assay for T cell cytotoxicity. *J. Immunol. Methods*. 58:225.
10. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogenic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47:63.
11. Bosma, G. C., R. P. Custer, and M. J. Bosma. 1983. A severe combined immunodeficiency mutation in the mouse. *Nature (Lond.)*. 301:527.
12. Coffman, R. L., and I. L. Weissman. 1981. A monoclonal antibody that recognizes B cells and B cell precursors in mice. *J. Exp. Med.* 153:269.
13. Symington, F. W., B. Subbarao, D. E. Mosier, and J. Sprent. 1983. Lyb 8.2: A new B cell antigen defined and characterized with a monoclonal antibody. *Immunogenetics*. 16:381.

14. Forman, J., and E. S. Vitetta. 1975. Absence of H-2 antigens capable of reacting with cytotoxic T cells on teratoma line expressing a T/t locus antigen. *Proc. Natl. Acad. Sci. USA*. 72:3661.
15. Bosma, M. J., G. C. Bosma, and J. L. Owen. 1978. Prevention of immunoglobulin production by allotype-dependent T cells. *Eur. J. Immunol.* 8:562.
16. Pravtcheva, D., A. B. DeLeo, F. H. Ruddle, and L. J. Old. 1981. Chromosome assignment of tumor-specific antigen of 3-methylcholanthrene-induced mouse sarcoma. *J. Exp. Med.* 154:964.
17. DuBois, G. C., L. W. Law, and E. Appella. 1982. Purification and biochemical properties of tumor-associated transplantation antigens from methylcholanthrene-induced murine sarcomas. *Proc. Natl. Acad. Sci. USA*. 79:7669.
18. Flood, P. M., A. B. DeLeo, L. J. Old, and R. K. Gershon. 1983. Relation of cell surface antigen on methylcholanthrene-induced fibrosarcomas to immunoglobulin heavy chain complex variable region-linked T cell interaction molecules. *Proc. Natl. Acad. Sci. USA*, 80:1683.
19. Trowbridge, I. S., P. Ralph, and M. J. Bevan. 1975. Differences in the surface proteins of mouse B and T cells. *Proc. Natl. Acad. Sci. USA*. 72:157.
20. Trowbridge, I. S., and C. Mazauskas. 1976. Immunological properties of murine thymus-dependent lymphocyte glycoproteins. *Eur. J. Immunol.* 6:557.
21. Michaelson, J., M. Scheid, and E. A. Boyse. 1979. Biochemical features of Ly-5 alloantigen. *Immunogenetics*. 9:193.
22. Scheid, M. P., and D. Triglia. 1979. Further description of the Ly-5 system. *Immunogenetics*. 9:423.
23. Omary, M. B., I. S. Trowbridge, and M. P. Scheid. 1980. T200 cell surface glycoprotein of the mouse. Polymorphism defined by the Ly-5 system of alloantigens. *J. Exp. Med.* 151:1311.
24. Siadak, A. W., and R. C. Nowinski. 1980. Identification of Ly-5 and T-200 antigens on identical cell surface proteins. *J. Immunol.* 125:1829.
25. Trowbridge, I. S., and M. B. Omary. 1981. Molecular complexity of leukocyte surface glycoproteins related to the macrophage differentiation antigen MAC-1. *J. Exp. Med.* 154:1517.
26. Coffman, R. L., and I. L. Weissman. 1981. B220: a B cell-specific member of the T200 glycoprotein family. *Nature (Lond.)*. 289:681.
27. Tung, J. S., M. P. Scheid, M. A. Pierotti, U. Hammerling, and E. A. Boyse. 1981. Structural features and selective expression of three Ly-5⁺ cell-surface molecules. *Immunogenetics*. 14:101.
28. Sarmiento, M., M. R. Loken, I. S. Trowbridge, R. L. Coffman, and F. W. Fitch. 1982. High molecular weight lymphocyte surface proteins are structurally related and are expressed on different cell populations at different times during lymphocyte maturation and differentiation. *J. Immunol.* 12:1676.
29. Ralph, S. J., and M. V. Berridge. 1984. Expression of antigens of the "T200" family of glycoproteins on hemopoietic stem cells: evidence that thymocyte cell lineage antigens are represented on T200. *J. Immunol.* 132:2510.
30. Tung, J. S., M. C. Deree, and E. A. Boyse. 1984. Evidence that Ly-5 product of T and B cells differ in protein structure. *Immunogenetics*. 19:149.
31. Scheid, M. P., K. S. Landreth, J. S. Tung, and P. W. Kincade. 1982. Preferential but nonexclusive expression of macromolecular antigens on B-lineage cells. *Immunol. Rev.* 69:141.
32. Morrison, M. H., W. G. Charey, and W. J. Esselman. 1984. Molecular weight and charge heterogeneity of Thy-1 glycoprotein in different populations of T cells. *Mol. Immunol.* 21:405.

33. Cowing, C., and J. Chapdelaine. 1983. T cells discriminate between Ia antigens expressed on allogenic accessory cells and B cells. A potential function for carbohydrate side chains on Ia molecules. *Proc. Natl. Acad. Sci. USA.* 80:6000.
34. Eardley, D. D., F. W. Shen, H. Cantor, and R. K. Gershon. 1979. Genetic control of immunoregulatory circuits. Genes linked to the Ig locus govern communication between regulatory T cell sets. *J. Exp. Med.* 150:44.
35. Yamauchi, K., D. Murphy, H. Cantor, and R. K. Gershon. 1981. Analysis of antigen-specific, Ig-restricted cell-free material made by I-J⁺ Ly-1 cells (Ly-1 TsiF) that induces Ly-2⁺ cells to express suppressive activity. *Eur. J. Immunol.* 11:905.
36. Sunday, M., B. Benacerraf, and M. E. Dorf. 1981. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. VIII. Suppressor cell pathways in cutaneous sensitivity responses. *J. Exp. Med.* 153:811.
37. Minami, M., I. Aoki, N. Honji, C. R. Waltenbaugh, and M. E. Dorf. 1983. The role of I-J and Igh determinants on F₁-derived suppressor factor in controlling restriction specificity. *J. Exp. Med.* 158:1428.
38. L'Age-Stehr, J. 1981. Priming of T helper cells by antigen-activated B cells. B cell-primed Lyt-1⁺ helper cells are restricted to cooperate with B cells expressing the IgVh phenotype of the priming B cells. *J. Exp. Med.* 153:1236.
39. Nutt, N., J. Haber, and H. H. Wortis. 1981. Influence of Igh-linked gene products on the generation of T helper cells in the response to sheep erythrocytes. *J. Exp. Med.* 153:1225.
40. Hetzelberger, D., and K. Eichmann. 1978. Recognition of idiotypes in lymphocyte interactions. 1. Idiotypic selectivity in cooperation between T and B lymphocytes. *Eur. J. Immunol.* 8:846.