Immunogenetics 20: 47–56, 1984

Immuno-genetics

Studies of the Mouse *Ly-6* Alloantigen System

II. Complexities of the *Ly-6* Region

Shoji Kimura*, Nobuhiko Tada†, Yen Liu-Lam, and Ulrich Hämmerling

Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021

Abstract. The discovery of several monoclonal antibodies provided the impetus to revisit the Ly-6 group of antigens. Our serological data point to the existence of at least five separate Ly-6 antigens. They are distinguished by the patterns of their tissue expression as (1) the classical Ly-6 alloantigen of peripheral lymphocytes (Ly-m6.2A), (2) a bone marrow cell-restricted antigen (Ly-m6.2B), (3) an antigen shared by bone marrow cells and peripheral lymphocytes (Ly-m6.2C, possibly identical with H9/25), (4) an antigen expressed on bone marrow cells, thymocytes, and peripheral lymphocytes (Ly-m6.2D), and (5) an antigen occurring exclusively on lymphoblasts (Ly-m6.1E, similar to Ala-1). ThB is a sixth distinct antigen of the group. The assumption that separate antigens exist is supported by distinctive distribution patterns in normal and neoplastic tissues. The genes controlling Ly-6 antigens are closely linked, as they are transmitted as two haplotypes only. One incidence of a crossover within the *Ly-6* region was observed: the Ly-6B.2 alloantigen was expressed in NZB mice, which type Ly-6.1 for other Ly-6 specificities.

Introduction

Some years ago three groups reported the discovery of very similar, genetically linked lymphocyte antigens, Ly-6 (McKenzie et al. 1977), Ala-1 (Feeney and Hämmerling 1976), and Ly-8 (Frelinger and Murphy 1976). These antigens were defined by alloantisera raised in a number of strain combinations and were found to be remarkably similar to one another in their strain distribution patterns and largely overlapping in their tissue distribution, although one of them, Ala-1, was

* *Present address:* Dept. of Pathology, Wakayama Medical College, 7-Ban-cho-7, Wakayama, Japan.
† *Present address:* Dept. of Pathology, Tokai University School of Medicine, Boseidai, Isehara, Kanagawa, Japan.

originally defined as an antigen predominating on activated lymphocytes. Because alloantisera are complex and not always reproducible as monospecific antibodies, the Ly-6 antigen was regarded as a single antigen system with several incompletely defined determinants. This view was supported by genetic evidence that showed marked linkage disequilibrium for *Ly-6* and *Ala-1* alleles in an extensive backcross breeding experiment (Feeney 1978). In addition, two nonlymphoid antigens, DAG (Sachs et al. 1973) and REN-1 (Halloran et al. 1978), and one lymphoid antigen, ThB (Eckhardt and Herzenberg 1980), were described, the controlling loci of which were inseparable from Ly-6. Additional Ly-6-like antigens emerged after the introduction of hybridoma technology. Takei and co-workers (1980) described the H9/25 antigen, which is based on a murine monoclonal antibody. Our work described previously (Kimura et al. 1980) and in the present report has given rise to several more monoclonal antibodies with partially distinctive reactivities. The availability of these resources warrants the re-examination of the Ly-6 antigens. The new serological data presented in this paper indicate that the *Ly-6* antigen system may comprise a group of antigens controlled by a cluster of closely linked genes.

Materials and Methods

Mice. Mice which have the suffix J and CXB recombinant inbred strains were purchased from the Jackson Laboratory, Bar Harbor, Maine. Other strains of mice were obtained from colonies maintained by Dr. E.A. Boyse at the Memorial Sloan-Kettering Cancer Center, New York. NZB/BIScr and NZB/Minnesota were supplied by Ms. Erin McKaig, Scripps Clinic and Research Foundation, San Diego, California, and Ms. June Smith, University of Minnesota, respectively.

Monoclonal antibodies. Monoclonal antibodies used in this study are listed in Table 1. Cell fusions were performed essentially according to the procedures of Köhler and Milstein (1975) as modified by us (Kimura et al. 1980a). High-titered sera of ascitic fluids used throughout this study were obtained by growing hybridoma cells intraperitoneally in appropriate F_1 mouse strains or Swiss nude mice. Immunoglobulin classes or subclasses of monoclonal antibodies were determined by immunodiffusion according to the method of Ouchterlony and Nilsson (1978) using monospecific rabbit antimouse immunoglobulin sera obtained from Litton Bionetics (Kensington, Maryland). The hybridoma cell lines used in this study are available to interested investigators.

Tumor cell lines. Tumor cell lines and their origin and characteristics are described in Table 2.

Table 1. A list of hybridoma cell lines used in this study

Clone	Immunization	Specificity	Ig class
S8.106	C3H/An anti-B6-H-2^{k}*	Ly-m6.2A	IgG2a, k
SK38.86	A-Thy-1.1 anti-K36	Ly-m6.2B	IgG2a, k
SK140.112	CBA/HN anti-C57BL/6*	Ly-m6.2C	IgM, k
SK142.446	HRS/J anti-C57BL/6*	Ly-m6.2C	IgM, k
SK142.476	HRS/J anti-C57BL/6*	Ly-m6.2D	IgM, k
SK70.36	C57BL/6 anti-BALB/c†	Ly-m6.1E	?
SK70.94	C57BL/6 anti-BALB/c†	Ly-m6.1E	IgG2a, k

* Normal spleen cells.
† Con A-activated T-cell blasts.

Cytotoxicity assay. Cytotoxicity assays were performed as described previously (Kimura et al. 1980a).

Preparation of activated lymphocytes. Spleen cells were cultured in vitro with concanavalin A (Con A) (2 μg/ml) for 2–3 days. Dead cells were removed from Con A-stimulated cultures by bovine serum albumin (BSA) gradient centrifugation.

Inhibition of biotin-labeled antibodies by unlabeled antibodies. Biotinylation of antibodies was performed according to the method described by Goding (1980). Briefly, crude immunoglobulin fractions prepared by 40% saturated ammonium sulfate precipitation were dialyzed against 0.1 *M* $NaHCO_3$. They were mixed with biotin succinimide ester (Sigma, St. Louis, Missouri) in dimethylsulfoxide and incubated for 4 h at room temperature. The conjugates were dialyzed overnight against phosphate-buffered saline (PBS) containing 0.1% sodium azide.

For the binding inhibition assay, the cells were first incubated with a saturating amount of unlabeled antibody on ice in the presence of sodium azide and then washed thoroughly with PBS. The washed cells were incubated with biotin-coupled antibody, washed, and mixed with fluoresceinated-avidin (Sigma, St. Louis, Missouri). The stained cells were then analyzed in a fluorescence-activated cell sorter (FACS) IV system.

Results

Tissue distribution of Ly-m6 alloantigens. Seven different monoclonal antibodies were derived from a series of immunizations as specified in Table 1. They were tested by direct cytotoxicity assay on cells of different lymphoid organs. The emerging tissue distribution patterns permitted the assignment of each antibody to one of five distinct groups defining five separate antigens. Thus, for solely housekeeping reasons these alloantigens were provisionally named Ly-m6A, B, C, D, and E. The tissue distribution patterns are summarized in Table 3. The Ly-6A antigen distribution resembles strikingly that described by McKenzie and co-workers (1977) with alloantiserum for Ly-6. Ly-m6A may therefore represent the classic *Ly-6* antigen system. We previously described a detailed account of our serological studies with a number of Ly-m6A monoclonal antibodies (Kimura et al. 1980b), including the clone S8/106 used in the present report. Ly-m6B is a new specificity which occurs exclusively on bone marrow cells and on a small proportion (5%) of lymph node and spleen cells. (This proportion is at the limit of the accuracy of the cytotoxicity assay and the results therefore are ambivalent.) A monoclonal antibody with a reactivity pattern comparable to that of the Ly-m6B antibody has not been reported before. Ly-m6C antibody produces a tissue distribution pattern similar to that of Ly-m6A, but in contrast to this classical Ly-6 antibody, it reacts strongly with bone marrow cells. The pattern is similar to the one reported for the rat antibody H9/25 by Takei and co-workers (1980). The fourth antibody, Ly-m6D, is again similar to the classical Ly-6A antibody, showing, however, additional reactivity with thymus cells. The last group, Ly-6E, comprises two monoclonal antibodies that react almost exclusively with Con A-activated T-cell blasts. On this score, Ly-6E antigen, defined by the two monoclonal antibodies SK70.36 and SK70.94, corresponds to Ala-1 described by Feeney and Hämmerling (1976).

To confirm the observations of differential tissue expression and to assess the quantitative relationships between different Ly-m6 alloantigens in different lymphoid organs, we performed quantitative absorption experiments. As shown in Figure 1 A–E, the absorption capacity of the cells from different lymphoid organs

Table 2. Expression of Ly-m6 alloantigens on established cell lines*

(1) Ly-m6.2 alloantigens (A–D)

	Origin	Cell type	Ly-m6.2A	Ly-m6.2B	Ly-m6.2C	Ly-m6.2D
BW5147	AKR	T cell	+	−	+	+
K36	AKR	T cell	+	+	+	+
EL-4	C57BL	T cell	+	−	−	+
EL-4Fa	C57BL/6	T cell	−	−	−	+
ERLD	C57BL/6	T cell	+	−	N. D.	N. D.
E♂G2	C57BL/6	T cell	+	−	N. D.	N. D.
B6RVTCI	C57BL/6	T cell	+	−	−	+
B6RV1	C57BL/6	T cell	+	−	−	+
B6RV2	C57BL/6	T cell	+	−	−	+
RBL-5	C57BL/6	T cell	−	−	N. D.	N. D.
I.29	I/St	B cell	+	−	N. D.	N. D.
L1210	DBA/2	B cell	+	−	N. D.	N. D.
70Z/3	(C57BL/6 × DBA/2)F_1	Pre-B cell	−	−	−	−
93-4	C57BL/6	Pre-B cell	−	−	−	+
R453	C57BL/6	Promyelocyte	+	−	N. D.	N. D.
P388D1	DBA/2	Macrophage	+	−	−	+
P815	DBA/2	Mastocyte	+	−	−	+
416B	(C57BL/6 × DBA/2)F_1	Stem cell	−	−	−	−
SK70.94	(C57BL/6-Lyt-1.1 × BALB/c)F_1	Myeloma	−	−	+	−
K75.22	(C57BL/6-*H-2*^{k} × BALB/c)F_1	Myeloma	−	−	+	−

(2) Ly-m6.1E alloantigen

	Origin	Cell type	Ly-m6.1E
ASL1	A	T cell	−
RADA1	A	T cell	−
YAC-1	A	T cell	−
RL♂1	BALB/c	T cell	−
RL♀4	BALB/c	T cell	−
RL♀8	BALB/c	T cell	−
BALBRVA	BALB/c	T cell	−
BALBVRC	BALB/c	T cell	−
BALBRVE	BALB/c	T cell	−
BALBRV1	BALB/c	T cell	−
BALBRV4	BALB/c	T cell	−
LSTRA	BALB/c	T cell	−
MOPC70A	BALB/c	Myeloma	−
MOPC104E	BALB/c	Myeloma	−
NS-1	BALB/c	Myeloma	−
SP2/0	BALB/c	Myeloma	−
C1.18	C3H	Myeloma	−
18-4	BALB/c	Pre-B cell	−
Pu5	BALB/c	Macrophage	−
HFL/bB^2	BALB-*H-2*^{b}	Erythroleukemia	−
HFL/bC^4	BALB-*H-2*^{b}	Erythroleukemia	−
DS-19	DBA/2	Erythroleukemia	−
MethA	BALB/c	Fibrosarcoma	+
CMS4	BALB/c	Fibrosarcoma	+
CMS17	BALB/c	Fibrosarcoma	+
C14	BALB/c	Fibrosarcoma	+
DMS4	BALB/c	Fibrosarcoma	+

* Determined by direct cytotoxicity assay.

Table 3. Tissue distribution of a group of Ly-m6 alloantigens

Antigen	Thymus	Lymph node	Spleen	Bone marrow	Activated lymphocytes*
Ly-m6.2A	5†	60–70	50–60	5	100
Ly-m6.2B	0	5	5	50–70	0
Ly-m6.2C	0	30–40	20–30	50–70	40–50
Ly-m6.2D	50–80	70	60	10–20	100
Ly-m6.1E	5	5	5	5	80–90

* Splenic lymphocytes were cultured in vitro with Con A (2 μg/ml) for 2–3 days. Viable cells were obtained by BSA gradient centrifugation from harvested cells.

† Determined by direct cytotoxicity assay using rabbit serum as complement. Values indicated maximum percent cytotoxicity calculated by the formula $\frac{a-b}{c} \times 100$, where a is the percentage of cell killed by antibody and complement and b is the percentage of cells killed by complement alone, and c is the percentage of viable cells in the complement control.

agrees in each case with the estimated antigen representation gleaned from the direct cytotoxicity assays.

Genetics of Ly-m6 alloantigens. Ly-m6A–E antibodies were tested on suitable target cells of a variety of mouse strains including CXB recombinant inbred (RI) strains. All five antibody types show essentially the same strain distribution (Table 4) (note that Ly-m6E detects the *Ly-m6.1* allele, whereas Ly-m6A-D antibodies detect the *Ly-m6.2* allele). The only discordant reaction occurred in NZB and related strains, which type positive with Ly-m6B and Ly-m6E, but are unreactive with Ly-m6A, C, and D. This reaction indicates a natural recombination in the NZB strains between the *Ly-m6B* locus and the other *Ly-6* loci. The seven CXB RI strains tested display only two *Ly-6* haplotypes; they are either Ly-m6ABCD$^+$E$^-$ or Ly-m6ABCD$^-$E$^+$. Hence, no recombination occurred in the *Ly-6* region during derivation of the strains.

Linkage analysis of genes controlling Ly-m6 alloantigens. The strain distribution pattern of Ly-m6 alloantigens (Table 4) strongly suggests that *Ly-m6* genes are either identical or very closely linked to one another. To confirm the linkage of *Ly-m6* genes, we tested (A.TL$^-$ × C57BL/6)F$_1$ × A/J backcross mice for Ly-m6A–D expression. No recombinant mouse in 112 backcross mice was found. The typing of (BALB/c × C57BL/6)F$_2$ mice with Ly-m6.2A- and Ly-m6.1E-specific antibodies revealed that the ratio of progeny mice to phenotypes fit the theoretical Mendelian ratios of allelic traits quite well: 30 Ly-m6.1E$^+$ Ly-m6.2A$^+$; 17 Ly-m6.1E$^+$ Ly-m6.2A$^-$; 14 Ly-m6.1E$^-$ Ly-m6.2A$^+$; and no Ly-m6.1E$^-$ Ly-m6, 2A$^-$ mice among the 61 animals tested.

Representation of Ly-m6 alloantigens on tumor cell lines. Ly-m6-specific monoclonal antibodies were tested by cytotoxicity assay on a battery of established tumor cell lines with T-cell, B-cell, or monocyte characteristics. The results are summarized in Table 2. We found that the reaction pattern of each antibody (except Ly-m6E antibody) generally reflects the distribution of the respective antigen in normal tissues. For instance, the widest distribution is seen with Ly-m6D antibody, and the

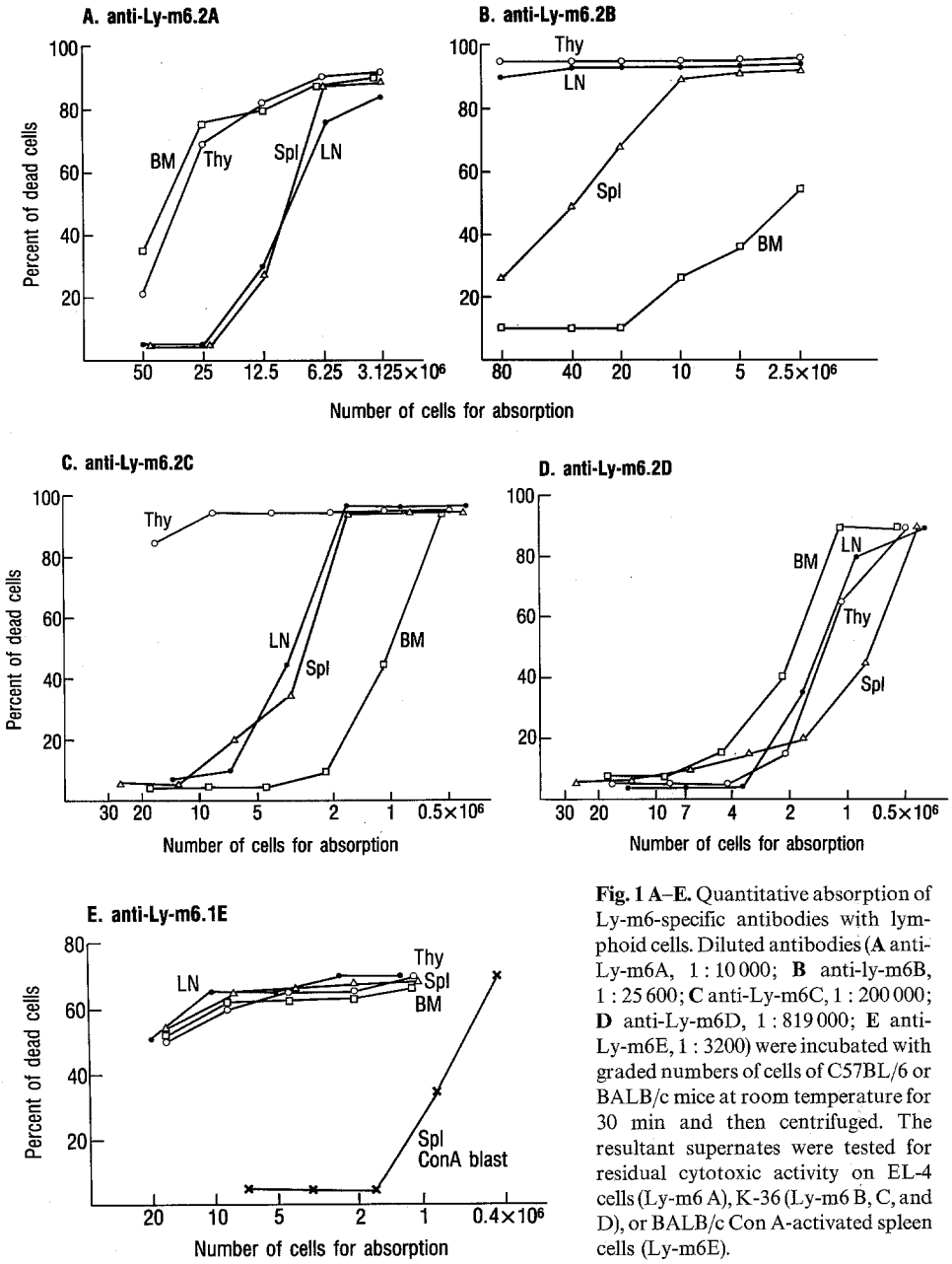

Fig. 1 A–E. Quantitative absorption of Ly-m6-specific antibodies with lymphoid cells. Diluted antibodies (**A** anti-Ly-m6A, 1 : 10 000; **B** anti-ly-m6B, 1 : 25 600; **C** anti-Ly-m6C, 1 : 200 000; **D** anti-Ly-m6D, 1 : 819 000; **E** anti-Ly-m6E, 1 : 3200) were incubated with graded numbers of cells of C57BL/6 or BALB/c mice at room temperature for 30 min and then centrifuged. The resultant supernates were tested for residual cytotoxic activity on EL-4 cells (Ly-m6 A), K-36 (Ly-m6 B, C, and D), or BALB/c Con A-activated spleen cells (Ly-m6E).

most restricted pattern occurs with Ly-m6B alloantibody. It is interesting to note that there is no lymphoid tumor reactive with Ly-m6E antibody, whereas all fibrosarcomas tested are positive. The latter cells are negative for the other Ly-m6 antibodies. The segregation of Ly-m6 specificities among the tumor cells suggests that five separate alloantigens are involved.

Spatial relationship of Ly-m6.2 determinants on the cell surface. The location of Ly-m6 determinants relative to one another at the cell surface was probed by mutual binding-interference assays. In these assays pairs of antibodies are applied to cells. One of the antibodies is tagged with biotin which allows its visualization with fluoresceinated avidin. The second unlabeled antibody is applied in different concentrations, and the degree of inhibition of uptake of the first antibody is monitored by fluorescence flow cytometry. As indicated in Figure 2 Ly-m6A and Ly-m6D antibodies mutually block each other. This suggests that the Ly-m6A and Ly-m6D determinants exist in close proximity to each other at the cell surface. Because this blockade is mutual, sterical interference rather than differences in affinity appears to be the cause of inhibition. Unidirectional inhibition, such as observed for Ly-m6A antibody towards biotinylated Ly-m6B antibody, is difficult to interpret.

Discussion

Several observations support our hypothesis that Ly-6 antigens constitute a group of antigens controlled by a multigene family. At the genetic level evidence for separate genes is scant. Backcross breeding experiments have consistently failed to

Table 4. Strain distribution of Ly-m6 alloantigens*

Strains	Ly-m6.2A	Ly-m6.2B	Ly-m6.2C	Ly-m6.2D	Ly-m6.1E
AKR, BDP/J, BUB/BnJ, C57BL/6, C57BL/KsJ, C57BL/10J, B10.S, C57BR/cdJ, C57L/J, C58, DBA/1J, DBA/2J, GR/A, HSFS/N Swiss, I/St, LG/J, LP/J, MA/MyJ, P/J, PL/J, RF/J, SJL/J, SM/J, SWR/J, 129, CXB RI strains: D, E, I, K	+	+	+	+	−
A, AU/SsJ, BALB/c, C3H/An, C3Hf/BI, C3H/Fg, C3H/HeJ, CBA/J, CBA/CaJ, CBA/H-T6J, CBA/HN, CE/J, HRS/J, RIIIS/J, SEA/GnJ, SEC/1ReJ, ST/bJ, STS/A, CXB RI strains: G, H, J	−	−	−	−	+
NZB/BLnJ, NZB/BLScr, NZB/Minnesota, NZW/LacJ	−	+	−	−	+

* Determined by cytotoxicity assay using appropriate target cells (i. e., A, lymph node cells; B and C, bone marrow cells; D, thymocytes; and E, Con A-activated cells).

separate the controlling genes for Ly-6 antigens as well as for ThB and H9/25 (Eckhard and Herzenberg 1980, Takei 1980), suggesting control by a single *Ly-6* gene. However, the discovery of the naturally occurring segregation of the Ly-6B antigen in NZB mice suggests that the *Ly-6B* gene is separate. To date, the chromosomal association of Ly-6 is still uncertain. Meruelo and colleagues (1982) favor a location on chromosome 2 centromeric to Ly-11 and H-3. Other investigators have suggested linkage to Thy-1 on chromosome 9 (Horton and Hetherington 1980).

Also supporting the argument in favor of the existence of separate Ly-6 antigens is their distinctive distribution in normal and malignant tissues. Although these are soft criteria, mutual exclusion of two Ly-6 antigens from two different cell types (such as observed, for instance, for Ly-6B of bone marrow and Ly-6E of activated T and B cells) is more in line with the existence of two separate antigens than of one antigen occurring in different serological states.

The third supporting argument stems from immunochemical analyses. According to reports, Ly-6A, ThB, and H9/25 have molecular weights of 33 500, 16 000, and 12 000–15 000, respectively, and these proteins are therefore most likely encoded independently (Matossian-Rogers et al. 1982, Takei 1982). However, the

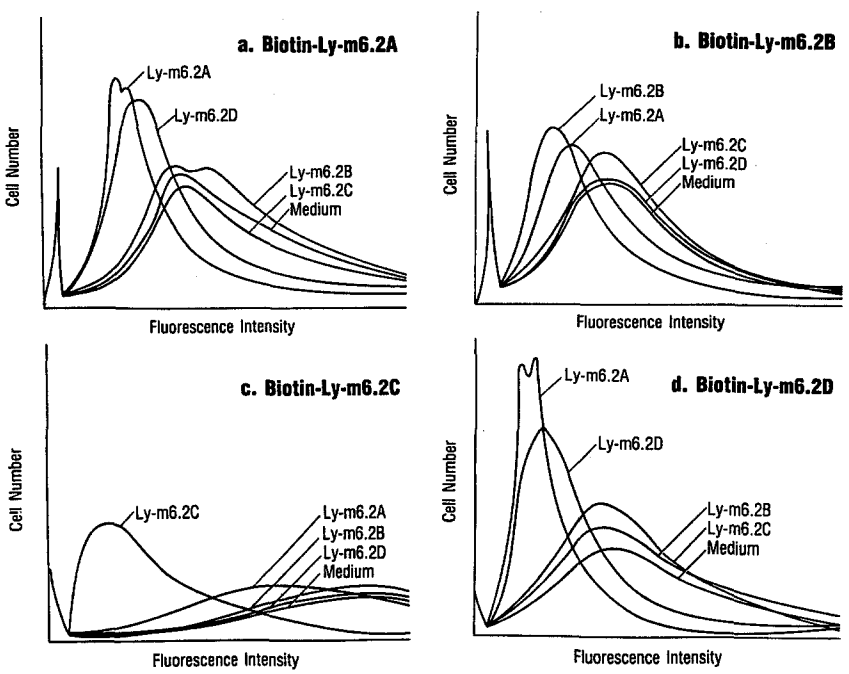

Fig. 2a–d. Inhibition of binding of biotin-labeled Ly-6.2-specific antibodies by unlabeled Ly-m6.2-specific antibodies. K36 cells, an AKR T-cell tumor, were incubated with unlabeled Ly-m6.2-specific antibodies or medium (control) for 30 min on ice. After washing, the cells were incubated for 30 min on ice with different biotin-labeled Ly-m6.2-specific antibodies (see panels **a–d**), then washed and mixed with fluoresceinated-avidin for 30 min on ice. All reactions were done in the presence of 0.1% NaN_3. The stained cells were analyzed by FACS IV system.

possibility must be considered that a single protein carrying two or more Ly-6 determinants but occurring in different sizes exists. Such differences in size expression are known to occur in the Ly-5/T200 antigen (Tung et al. 1981). The *Ly-5* system displays, however, no separation of antigenic determinants among the different cell types. T200 and Ly-5 determinants coexist on the same cell types. Another precedent would be Thy-1, where several molecular forms are created by posttranslational events (Barclay et al. 1976). The decisive biochemical experiments would be sequential immunoprecipitation as applied to the *TL* antigen system (McIntyre et al. 1982). These are now in progress.

In summary, our serological data, although not conclusive, favor the existence of at least six Ly-6-like antigens (i. e., Ly-6A, Ly-6B, Ly-6C or H9/25, Ly-6D, Ly-6E or Ala-1 and ThB). We have reservations about Ly-6D being an independent antigen, as the differences to Ly-6A are subtle. The notion that the *Ly-6* region comprises a group of genes has been expounded by others. Auchincloss and co-workers (1981) and Takei and Horton (1981) reported the existence of distinct lymphocyte antigens controlled by genes linked to Ly-6. Our initial interpretation of serological data obtained with Ala-1 antisera (Feeney and Hämmerling 1976) was in favor of the existence of such an antigen separate from Ly-6. However, failure to separate the respective genes and difficulties in reproducing the antisera led to the consensus that Ala-1 and Ly-6 were one and the same (Feeney 1978, Horton and Sachs 1979). However, in view of the data obtained with monoclonal antibodies this decision may have been premature, and it is now more likely that Ala-1 is a separate antigen. In conclusion, the *Ly-6* antigen complex is one more example that supports the general rule that the genes encoding Ly antigens are organized into several multigene families. To date, nine clusters have been defined on seven chromosomes (Tada et al. 1981), which cumulatively contain the genes controlling the majority (95%) of the serologically defined lymphocyte surface antigens.

Acknowledgments. The authors are indebted to Ms. Denise Taylor for the preparation of the manuscript. This work has been supported in part by the following grants: CA-07848, CA-37937, and AI-19682 from the National Institutes of Health.

References

Auchincloss, H., Jr., Ozato, K., and Sachs, D. H.: Two distinct murine differentiation antigens determined by genes linked to the *Ly-6* locus. *J. Immunol. 127:* 1839–1843, 1981

Barclay, A. N., Letarte-Muirhead, M., Williams, A. H., and Fanlkes, R. A.: Chemicals characterization of the Thy-1 glycoproteins from the membranes of rat thymocytes and brain. *Nature 263:* 563–567, 1976

Eckhardt, L. A. and Herzenberg, L. A.: Monoclonal antibodies to ThB detect close linkage of *Ly-6* and a gene regulating ThB expression. *Immunogenetics 11:* 275–291, 1980

Feeney, A. J.: Expression of Ly-6 on activated T and B cells: possible identity with Ala-1. *Immunogenetics 7:* 537–543, 1978

Feeney, A. J. and Hämmerling, U.: Ala-1: A murine alloantigen of activated lymphocytes. I. Serological analysis of mitogen-stimulated T and B cells. *Immunogenetics 3:* 369–379, 1976

Frelinger, J. A. and Murphy, D. B.: A new alloantigen, Ly-8, recognized by C3H anti-AKR serum. *Immunogenetics 3:* 481–487, 1976

Goding, J. W.: Antibody production by hybridomas. *J. Immunol. Methods 39:* 285–308, 1980

Halloran, P.F., Dutton, D., Chance, H., and Cohen, Z.: An Ly-like specificity with extensive nonlymphoid expression. *Immunogenetics 7:* 185–200, 1978

Horton, M.A. and Hetherington, C.M.: Genetic Linkage of *Ly-6* and *Thy-1* loci in the mouse. *Immunogenetics 11:* 521–525, 1980

Horton, A.M. and Sachs, J.A.: Identity of murine lymphocyte alloantigens DAG, Ala-1, Ly-8, and Ly-6? *Immunogenetics 9:* 273–280, 1979

Kimura, S., Tada, N., and Hämmerling, U.: A new lymphocyte alloantigen (Ly-10) controlled by a gene linked to the *Lyt-1* locus. *Immunogenetics 10:* 363–372, 1980a

Kimura, S., Tada, N., Nakayama, E., and Hämmerling, U.: Studies of the mouse Ly-6 alloantigen system. I. Serological characterization of mouse Ly-6 alloantigen by monoclonal antibodies. *Immunogenetics 11:* 373–381, 1980b

Köhler, G. and Milstein, C.: Continuous cultures of fused cells secreting antibody or predefined specificity. *Nature 256:* 495–497, 1975

Matossian-Rogers, A., Rogers, P., Ledbetter, J.A., and Herzenberg, L.A.: Molecular weight determination of two genetically linked cell surface murine antigens: ThB and Ly-6. *Immunogenetics 15:* 591–599, 1982

McIntyre, K.R., Hämmerling, U., Uhr, J.W., and Vitetta, E.S.: Structural analysis of thymus-leukemia (TL) antigens in the mouse. *J. Immunol. 128:* 1712–1717, 1982

McKenzie, I.F.C., Cherry, M., and Snell, G.D.: Ly-6.2: A new lymphocyte specificity of peripheral T-cells. *Immunogenetics 5:* 25–32, 1977

Meruelo, D., Offer, M., and Rossomando, A.: Evidence for a major cluster of lymphocyte differentiation antigens on murine chromosome 2. *Proc. Natl. Acad. Sci. U.S.A. 79:* 7460–7464, 1982

Ouchterlony, O. and Nilsson, L.A.: Immunodiffusion and immunoelectrophoresis. *In* D.M. Weir (ed.): *Handbook of Experimental Immunology*, third ed., Chapter 19, Blackwell, Oxford, 1978

Sachs, J.A., Huber, B., Pena-Martinez, J., and Festenstein, H.: Genetic studies and effect of skin allograft survival of DBA/2 DAG, Ly, and M-locus antigens. *Transplant. Proc. 5:* 1385–1387, 1973

Tada, N., Kimura, S., and Hämmerling, U.: Gene-cluster formation of mouse alloantigen loci. A survey with monoclonal antibodies. *In* G.J. Hämmerling, U. Hämmerling, and J. Kearney (eds.): *Monoclonal Antibodies and T Cell Hybridomas*, pp. 38–44, Elsevier/North Holland, Amsterdam, 1981

Takei, F.: Biochemical characterization of H9/25, an allospecificity encoded by the *Ly-6* region. *Immunogenetics 16:* 201–208, 1982

Takei, F. and Horton, M.F.: *Ly-6* region regulates expression of multiple allospecificities. *Immunogenetics 13:* 435–441, 1981

Takei, F., Galfre, G., Anderson, T., Lennox, E.S., and Milstein, C.: H9/25 monoclonal antibody recognizes a new allospecificity of mouse lymphocyte subpopulations: Strain and tissue distribution. *Eur. J. Immunol. 10:* 241–246, 1980

Tung, J.-S., Scheid, M.P., Pierotti, M.A., Hämmerling, U., and Boyse, E.A.: Structural features and selective expression of three Ly-5$^+$ cell surface molecules. *Immunogenetics 14:* 101–106, 1981

Received January 26, 1984; revised version received April 13, 1984