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A New Alloantigen, Ly-8, Recognized by C3H Anti-AKR Serum

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Abstract. A new membrane alloantigen, designated Ly-8.2, is defined by a C3H anti-AKR serum. The locus, *Ly-8*, which controls this determinant is not linked to *Thy-1*, *Ly-4*, *Ly-6*, *H-2*, albino (*c*), or brown (*b*). Ly-8.2 has a unique strain distribution, and appears to be present on both T and B lymphocytes.

Introduction

Anti-Thy-1 sera have been used widely as tools for detection of T cells in functional studies (Raff 1971). The possibility of other antibodies' contaminating the classical anti-Thy-1 sera has already been raised. Greaves and Raff (1971) noted that AKR anti-CBA sera contained antibodies which were cytotoxic to CBA B cells and were absorbed with CBA thymus, but not CBA brain cells. No further serological or genetic characterization of this specificity has been reported. Further, the presence of anti-immunoglobulin allotype has been reported (Baird *et al.* 1971), and anti-Ly-1 or anti-Ly-3 have been predicted in such sera on the basis of strain distribution. In this report, a new alloantigen system defined by the reactivity of a C3H anti-AKR serum with C57BL/10 (abbreviated to B10) tissue is described. The locus controlling this activity is not linked to *Thy-1*, *Ly-4*, *Ly-6*, *H-2*, albino (*c*), or brown (*b*). Determinants controlled by this system can be detected on both T and B lymphocytes. The locus is tentatively designated as *Ly-8*, the allele *Ly-8*^{b}, and the antigen it determines, Ly-8.2.

Materials and Methods

Mice were either raised in the animal facility at the University of Michigan's Department of Human Genetics, or purchased from the Jackson Laboratory, Bar Harbor, Maine, or from Cumberland Farms, Clinton, Tennessee. (BALB/c × C57BL/6) F_1 × BALB/c mice were generously provided by Ann Feeney, Memorial-Sloan Kettering Cancer Institute, New York.

Four anti-Thy-1 sera were used in this study. AKR/J anti-C3H/HeJ and A.AKR anti-A.AL (both anti-Thy-1.2), and C3H anti-AKR/J (anti-Thy-1.1) were produced according to the method of Reif and Allen (1966) by weekly injections of 10^7 thymocytes per recipient. AKR/Cum anti-AKR/J (anti-Thy-1.1) serum was provided by E. P. Blankenhorn, California Institute of Technology, Pasadena. This serum is produced in AKR sublines which differ at *Thy-1* (Acton *et al.* 1973). All antisera tested

were from large batches of pooled sera. A.AKR (*Thy-1*^{a}) and A.AL (*Thy-1*^{b}) are a congenic strain pair which differ only at *Thy-1*.

Anti-Ly-6.2 serum [(A × BALB/c) anti-CXBD] was provided by Dr. Marianna Cherry, Jackson Laboratory, Bar Harbor, Maine. Anti-Ly-4.2 [(SWR × BALB/c) anti-B10.D2] was prepared as described previously by Snell and coworkers (1973). Anti-H-2.13 serum was produced in (B10.BR × A.BY) F_1 by immunizing with B10.AKM tissue.

A two-stage dye exclusion test using rabbit complement was performed according to the method of Amos and coworkers (1969), as modified by Frelinger and coworkers (1974). Hemagglutination assays and in vitro absorption analyses were conducted as described previously (Frelinger *et al.* 1974, Stimpfling 1961). Direct plaqueforming cells were assayed by the method of Jerne and Nordin (1963). Splenic or lymph node T cells were purified on nylon wool columns (Julius *et al.* 1973).

Results and Discussion

Treatment of AKR lymph node or thymus cells with a C3H anti-AKR serum in the presence of rabbit complement results in 60 to 70 percent or 100 percent lysis, respectively. In contrast, 60 to 70 percent of B10 (C57BL/10SgSnSf) lymph node cells are killed by this antiserum, while less than 15 percent of B10 thymus target cells are lysed (Fig. 1). In order to determine whether two antibody populations are involved, an in vitro absorption analysis was conducted. Absorption with B10 or AKR thymus cleared all activity for B10 lymph node targets (Fig. 2). B10 thymus completely removed activity for B10 lymph node targets (top panel), while leaving reactivity for both AKR lymph node and thymus cells (bottom two panels). In no case did C3H cells remove any activity, suggesting that no autoantibody is being detected in this reaction. Absorbing with AKR/J lymph node cells gave results equivalent to those obtained with AKR thymus cells. These data suggest that this serum recognized two distinct determinants – one expressed only on AKR cells (Thy-1.1), the other shared by B10 and AKR (Ly-8.2). B10 kidney, liver, and brain did not absorb activity for B10 lymph nodes.

Since C3H and B10 share the same *Thy-1*^{b} allele, reactivity with B10 should not involve antibody directed against Thy-1.1 determinants. This conclusion is supported by the marginal direct reaction observed with B10 thymus target cells.

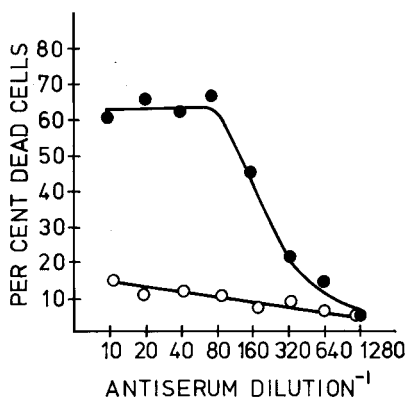

Fig. 1. Direct cytotoxic test of C3H anti-AKR: B10 lymph node lymphocytes ●—●; B10 thymus cells ○—○

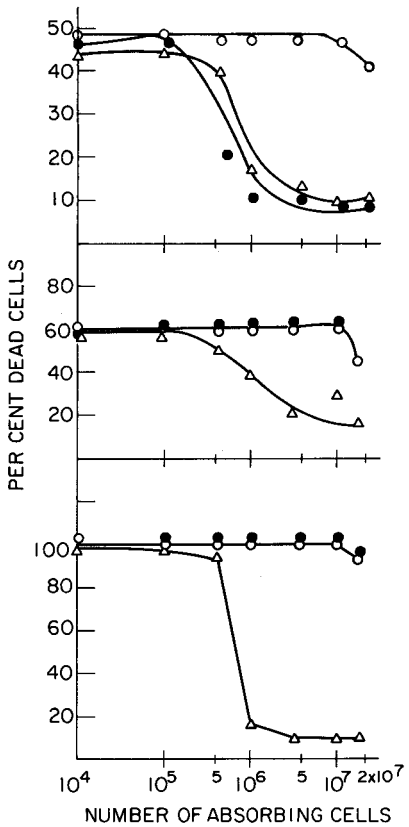

Fig. 2. Absorption analysis of C3H anti-AKR serum. Top panel shows the absorption of C3H anti-AKR diluted 1/100 by B10 ●—●, AKR △—△, or C3H ○—○ thymus cells for B10 lymph node targets. Center panel shows absorption of C3H anti-AKR by B10 ●—●, AKR △—△, or C3H ○—○ thymus cells for AKR lymph node target cells. Bottom panel shows the same absorbed sera tested with AKR thymus target cells

Thy-1 antigens normally are easily detected on this cell population. In addition, strain A.AKR thymus cells, which express Thy-1.1 determinants, fail to absorb reactivity for C57BL/10 lymph node cells. In order to verify that *Ly-8* and *Thy-1* are distinct genetic loci, a linkage test was performed. (AKR.M × C3H)F_1 mice were backcrossed to C3H mice, and the progeny were typed for *Thy-1*, *Ly-8*, and *H-2* (Table 1). AKR.M mice were used to provide an *H-2* marker. Of thirty-six backcross mice, 26 reacted with the C3H anti-AKR/J serum (anti-Thy-1.1, anti-Ly-8.2). This result clearly demonstrates the segregation of two unlinked genes. *Thy-1^b* homozygous animals were determined by a negative reaction with an AKR/Cum anti-AKR/J (anti-Thy-1.1) serum. Of sixteen *Thy-1^b* homozygotes, six were typed Ly-8.2. These data show that *Ly-8* and *Thy-1* are independent loci. Typing these same mice for H-2.13 (marker for *H-2D^q*) indicates that *Ly-8* is not linked to *H-2* (Table 1).

Table 1. Segregation of *Ly-8* and *Thy-1*, *H-2*

Loci Tested	Number of Mice In Each Class				Cross
	++	+−	−+	−−	
Thy-1 vs. *Ly-8*	(20)		6	10	(C3H × AKR.M) F_1 × C3H
H-2 vs. *Ly-8*	3	6	3	4	(C3H × AKR.M) F_1 × C3H

Table 2. Strain Distribution of *Ly-8*

Strain	*Thy-1*	C3HαAKR Ly-8.2	AKRαC3H Ly-8.1
A/WySnSf	*b*	−	(+)[a]
BALB/cJ	*b*	−	?
CBA/J	*b*	−	?
C57BL/10SgSnSf	*b*	+	?
C57BR/cdJ	*b*	+	?
C57L/J	*b*	+	?
C3H/HeSf	*b*	−	+
DBA/1Fs	*b*	−	?
DBA/2Fs	*b*	+	?
HTG/Sf	*b*	+	?
SJL/J	*b*	+	?
SM/J	*b*	+	?
SWR/J	*b*	+	?
A.AKR/Sf	*a*	(−)	+
AKR/J	*a*	+	−
BDP/J	*a*	?	±
BUB/J	*a*	?	−
PL/J	*a*	?	+
RF/J	*a*	?	+

[a] Deduced from reactivity of A.AKR.

Since the C3H anti-AKR serum used in this study contains anti-Thy-1.1 antibody, one can test anti-Ly-8 activity directly only against *Thy-1*^{b} strains. Similarly, one can test the reciprocal antiserum, AKR anti-C3H, only against *Thy-1*^{a} strains. These results are presented in Table 2. This strain distribution is unlike any other previously defined alloantigen system. Although we show in Table 2 the strain distribution of both antisera, we have no evidence to prove that the reciprocal sera recognize allelic products. We stress that, aside from the strain distribution shown here, all subsequent work was performed using the C3H anti-AKR serum. The specificity defined by this antiserum is designated Ly-8.2. The antigen recognized by the reciprocal serum may or may not be Ly-8.1.

The strain distribution of Ly-8.2 is similar but not identical to the distribution of Ly-4.2 (Snell *et al.* 1973) and Ly-6.2 (Cherry, personal communication). Two crosses were examined to determine possible linkage to either system. In the first cross, the progeny of a (C3H × B10)F_1 × C3H backcross were typed for Ly-8.2 and Ly-4.2. These loci clearly segregate independently (Table 3). In the second

Table 3. Segregation of Ly-8 and Ly-4, Ly-6, albinism (c) and brown (b)

Loci Tested	Number of Mice in Each Class				Cross
	++	+−	−+	−−	
Ly-4 vs. *Ly-8*	4	3	4	3	(B10 × C3H) F_1 × C3H
Ly-6 vs. *Ly-8*	5	3	5	7	(C57BL/6 × BALB/c) F_1 × BALB/c
c vs. *Ly-8*	6	5	4	5	(C57BL/6 × BALB/c) F_1 × BALB/c
b vs. *Ly-8*	2	2	2	2	(C57BL/6 × BALB/c) F_1 × BALB/c

cross, (BALB/c × C57BL/6)F_1 × BALB/c backcross animals were typed for Ly-8.2 and Ly-6.2. Again, no linkage is apparent (Table 3). The same mice were classified for coat color. No linkage to the albino (*c*) or brown (*b*) loci was evident. In all linkage tests, χ^2 analysis showed no significant deviation from 50 percent recombination (unlinked genes). These data suggest that *Ly-8* is not on chromosome 2 (*Ly-4*), chromosome 4 (*b*), chromosome 7 (*a*), chromosome 9 (*Thy-1*), or chromosome 17 (*H-2*).

The data thus far demonstrate that the C3H anti-AKR serum contains two antibodies, anti-Thy-1.1 and anti-Ly-8.2. Potentially, this serum could also contain antibody against Ly-1.2, Ly-3.1, Ly-6.2, and Ly-7.2. One could argue that Ly-8.2 merely redefines one of these systems, but this possibility appears unlikely for several reasons. First, we have shown that Ly-8.2 has a unique strain distribution, and that *Ly-8* and *Ly-6* segregate independently. Second, strains C3H and B10 (the typing strain for Ly-8.2) share Ly-3.2 and Ly-7.1 determinants. Finally, Ly-1.2 and Ly-3.1 are readily detectable on thymocytes, whereas Ly-8.2 demonstrates only marginal activity with this cell population. The reaction cannot be caused by anti-Ly-5, since B10, C3H, and AKR all share the Ly-5.1 specificity (Komoro *et al.* 1975).

No reaction was observed with the C3H anti-AKR serum in immunodiffusion tests against the donor serum. Further, the strain distribution of Ly-8.2 is unlike any previously defined allotype system. These observations suggest that the observed cytotoxic reactions are not the result of unknown antiallotype antibodies reacting directly with surface Ig of lymphocytes. Neither C3H anti-AKR nor AKR anti-C3H has detectable hemagglutinating activity for donor red blood cells.

The 70 percent of cytotoxicity observed against lymph node lymphocytes suggests that reactivity cannot be limited to either the T or B population. Since B10 thymus cells completely absorb for B10 lymph node cells and the serum was produced against thymus preparations, at least some cells in the thymus – presumably T cells – express Ly-8. Direct cytotoxic tests with nylon wool-purified T cells (approximately 80 percent Thy-1-positive) from either spleen or lymph node show 60 to 70 percent sensitive cells (Fig. 3). This antiserum also kills B10 plaque-forming cells. Mice were primed intraperitoneally five days before sacrifice with 0.1 ml of 10 percent burro red cells. Treatment of the in vivo-primed spleen with anti-Ly-8 and complement greatly reduced the number of direct plaque-forming cells (Table 4). Antiserum against Ly-4.2 – a known B-cell marker – also

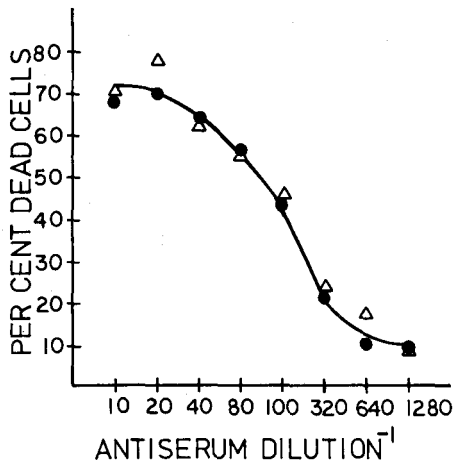

Fig. 3. Direct cytotoxic test of C3H anti-AKR on purified T cells. Spleen ●—●, or lymph node △—△ nylon wool-purified T cells tested with decreasing concentrations of C3H anti-AKR serum

Table 4. Effect of Anti-Ly-8 on B10 Direct Plaque-Forming Cells

Treatment	Pfc/10^6 Viable Cells
None	888
Anti-Thy-1.2 + c′	729
Anti-Ly-4.2 + c′	32
Anti-Ly-8.2 + c′	56

reduced the number of plaque-forming cells. Equal numbers of viable cells were tested in each case. These data clearly show that Ly-8 is expressed on both T cells and plaque-forming cells.

In order to test Ig-bearing cells directly, T-depleted B10 spleen cells were prepared by treatment with anti-Thy-1 and rabbit complement. The dead cells were removed by centrifugation on Lymphoprep, and the remaining cells were tested in direct cytotoxicity tests. This cell population is more than 80 percent Ig-positive by immunofluorescence. Anti-Ly-8.2 lyses approximately 60 percent of this cell population (Fig. 4). Anti-Thy-1.2 shows no detectable killing, demonstrating the absence of T cells in this preparation. Essentially all of these cells are sensitive to an appropriate anti-H-2 serum, showing that all the cells are susceptible to lysis. These results show that at least a portion of Ig-bearing cells express Ly-8 determinants.

We have presented evidence which defines a new alloantigen system, designated *Ly-8*. Formal genetic linkage studies have shown that *Ly-8* is not linked to *Ly-4*, *Ly-6*, *Thy-1*, or *H-2*. A distinct pattern of reactivity with a standard panel of strains further suggests that this system is unlike any other which has been well characterized on lymphocytes. Greaves and Raff (1971) demonstrated anti-B cell activity in an AKR anti-CBA serum, but failed to characterize this activity further. It is possible that the antigen studied by these investigators is identical to that

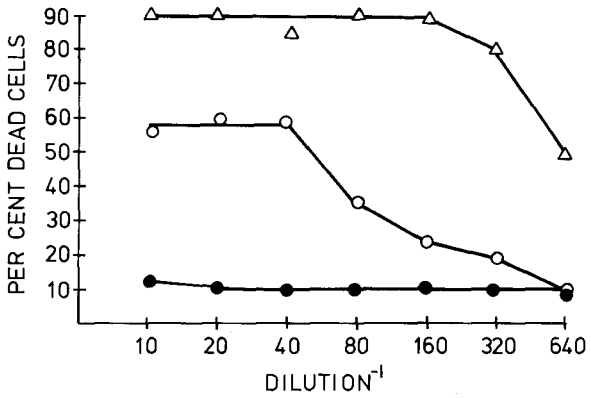

Fig. 4. Direct cytotoxic test of C3H anti-AKR on Thy-1-depleted B10.D2 spleen cells: C3H anti-AKR ○—○, anti-Thy-1.2 ●—●, anti-H-2.4 △—△

detected in the AKR anti-C3H serum, but such an association is purely conjectural. Antigens detected by the C3H anti-AKR serum are present on both T and B lymphocytes. Studies are currently underway to determine if this marker demarcates subsets in either T or B lymphocyte populations.

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