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Brief Communications

Igh-4d, a New Allotype at the Mouse IgG1 Heavy Chain Locus

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Allotypes (serologically distinct alleles) of mouse immunoglobulin (Ig) have been studied in detail using conventional serology (Herzenberg and Herzenberg 1978, Lieberman 1978) and, more recently, monoclonal antibodies (Oi and Herzenberg 1979). From these studies, it is clear that there is a wealth of genetic variation at the constant region loci. For example, allotypes have been discovered for Ig proteins of every heavy chain class and subclass except for IgG3. These alleles are assorted in various ways to form the constant region haplotypes of inbred laboratory mouse strains. The largest number of alleles have been defined at the *Igh-1* locus (IgG2a, see Green 1979 for nomenclature) while to date only two alleles have been defined for IgG1 (Igh-4). Here we describe a new IgG1 allotype, designated Igh-4d. This allotypic marker further defines the relationship of NZB (*Igh-n*), AL/N (*Igh-o*), AKR (*Igh-d*), and A/J (*Igh-e*) haplotypes.

The alloantiserum was generated by injecting AKR/J mice with an Igh-4a protein (anti-Igh-1b monoclonal antibody 4.7, Oi and Herzenberg 1979) coupled to KLH (Buttin et al. 1978). Specific antibody was purified by binding to 4.7 coupled to sepharose, and was radiolabeled while still bound to antigen as described by Herzenberg and Herzenberg (1978).

The radiolabeled AKR anti-4.7 was tested in the solid phase radioimmunoassay for direct binding to various immunoglobulins (Table 1). The antiserum reacted well with IgG1 proteins of both *a* and *b* haplotype origin, but not with proteins of other isotypes. When Fab and Fc fragments of IgG1 were tested, we found that although both Fab and Fc of 4.7 were reactive, only the Fc portions of other IgG1 proteins showed activity (Table 1). This indicates the presence of anti-idiotype and anti-Fc antibodies in our antiserum. Igh-4b protein MOPC 245T was able to completely inhibit the binding of the antiserum to Igh-4a proteins other than the immunogen, indicating that the anti-Fc reactivity detects determinants common to Igh-4a and Igh-4b proteins.

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Using a solid phase radioinhibition assay, we conducted a survey of the inbred mouse strains to determine the distribution of the IgG1 marker (Table 2). To avoid the anti-idiotype reactivity, an Igh-4b protein (MOPC-245T) was used for a plate coat. After 1 h the plates were rinsed well with phosphate-buffered saline plus 1% bovine serum albumin. Twenty microliters of centrifuged serum at various dilutions were added along with 20 µl of the ^{125}I-AKR anti-4.7. After 1 h incubation the wells were rinsed and counted. IgG1 levels of all Igh-non-b sera were quantitated in a similar assay using monoclonal antibody anti-Igh-4a 10.9 (Oi et al. 1978). This antibody detects an IgG1 determinant present in all Igh-non-b strains.

AKR (the donor strain, Igh-d) and NZB (Igh-n) sera did not react. Serum from the AL/N strain, as well as the Ig congenic line (CAL.20), previously classified as similar to AKR at the IgG loci, reacted with the antiserum. Sera from all other strains reacted, with a few strains showing less activity. These sera were shown to have lower levels of IgG1.

To map the genetic control of this marker on IgG1 proteins, we employed a congenic line, C57BL/6.Ign (Noel Warner, personal communication). This strain has the Igh haplotype of NZB imposed on a C57BL/6 background. Since the serum of C57BL/6.Ign behaves as NZB serum in our assay, the expression of this marker is genetically linked to the Igh chromosome region. We therefore have defined a new IgG1 allotype, Igh-4d, which lacks the specificity detected by this antiserum. This new specificity found on all Igh-4 proteins except those of Igh-4d origin, we designate Igh-4.3 (Table 3).

We have examined approximately 100 wild mouse sera, kindly provided by Dr. Jan Klein, Tübingen, F.R.G., for specificity 4.3. All sera possessed this specificity, indicating that *Igh-4*^{d} homozygotes are relatively rare in these outbred mice. Further allotypic analysis of these mice is being done with monoclonal antibodies (Huang et al. 1981).

It is interesting to examine the allotypic markers of the A/J (Igh-e), AKR (Igh-d), AL/N (Igh-o), and NZB (Igh-n) strains in light of what we know of heavy chain gene organization (Table 4). While the *d* and *e* haplotypes exhibit many similar specificities, they can now be distinguished at most of the polymorphic Ig loci. The AL/N and NZB strains each show a different combination of the *Igh-d* and *Igh-e* alleles. NZB appears similar to AKR at the *Igh-6, 5, 4* loci while resembling A/J at the downstream *Igh* loci. AL/N presents the converse situation.

Although recombination events have been sought in numerous backcrosses using inbred laboratory strains, they have not been found. Invariably such studies have employed Igh-a and Igh-b strain combinations (for which markers exist at either end of the *Igh* chromosomal region). The existence of two strains which resemble natural recombinants between *Igh-d* and *Igh-e* haplotypes in the region of IgG1 suggest that these strains and this chromosomal region may be of particular genetic interest.

The antiserum described here detects a new allotypic specificity, 4.3. This specificity is shared by all Igh-4 proteins, except those of AKR and NZB origin, and thus defines the new Igh-4d allotype. Using this antiserum, we have further defined the relationships between the *Igh-d, e, n,* and *o* haplotypes, with the *Igh-n* and *Igh-o* haplotypes resembling reciprocal natural recombinants of the *Igh-d* and *Igh-e* chromosomes.

Table 1. Reactivity of AKR anti-4.7 with myeloma and hybridoma proteins

	Isotype	Allotype	Reactivity
	IgG1	a, b	+
	IgG_{2a}	a, b	−
	IgG_{2b}	a, b	−
	IgG3		−
	IgM	a, b	−
	IgA	a, b	−
MOPC 245T	IgG1	b	+
	Fc		+
	Fab		−
4.7	IgG1	a	+
	Fc		+
	Fab		+

Table 2. Strain distribution of AKR anti-4.7 reactivity

Positive
A/J (*e*)*, A/S, ABP/Le, AL/N, (*o*)*, Au/SST, BALB/c, BAB/14, BDP/J, BUB/J, CAL/20, Castaneus, C3H, C57BI/6J, C57BL/10J, C57BL/KsJ, C57BR/CdJ, C57L/J, C58/J, CB/17, CBA/CaJ, CBA/J (*j*)*, CE/J (*f*)*, CSW, CWB/5, DBA/1J, DW/J, Fc/Le, FL/2Re, FS/Ei, Hook, HRS/J, Is/Camei, I/LnJ, Lt/ChRe, LP/J, Ma/MyJ, P/J, PL/J, RF/J, RIII/J (*g*)*, ROP/GNLe, SEA/J (*h*)*, SJL/J, SJA/9, St/DJ, SM/J, SWR/J, SEC/ReJ, Wa-Z, WB/ReJ, WC/Re, WH/Re, 129/J
Negative
AKR/J (d)*, AKR/Cm, NZB/BiNJ (n)*, C57BL/6-Ign

* Immunoglobulin type strains for allele indicated in parentheses.

Table 3. Igh-4 specificities

Type	Alleles	Specificities
BALB/c	*Igh-4*^{a} (*c, e, f, g, h, j, o*)	1 – 3
AKR/J	*Igh-4*^{d} (*n*)	1 – –
C57BL/10J	*Igh-4*^{b}	– 2 3

Table 4. Allotypes of *Igh-d, e, n,* and *o* haplotypes

Strain	*Igh* locus*	6	5		4	3	1	7	2
	Heavy chain	M	D	G3	G1	G2b	G2a	E	A
AKR		d	d		d	d	d		d/e
NZB		d	d		d	e	e		d/e
AL/N		e	e		a	d	d		d/e
A/J		e	e		a	e	e		d/e

* Allotypes at each locus were deduced from the following: Igh-6: Black et al. 1978, Kung et al., personal communication. Igh-5: Woods et al. 1980a, Woods et al 1980b. Igh-4: this paper and Herzenberg and Herzenberg 1978. Igh-3, 2, 1: Herzenberg and Herzenberg 1978. No differences between AKR and A/J have been reported for the Igh-2 proteins. Gene order is that deduced for BALB/c.

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