Population Genetics of the Heavy Chain Immunoglobulin Allotypes in the Rabbit[1] (36804)

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(Introduced by B. Cinader)

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Synthesis of the heavy immunoglobulin chain is controlled by two genes, one being in control of the variable, the second in control of the constant portion of the chain (1–3). Elucidation of the relation between the genes for constant and variable portions of the heavy chain and of the mechanism of assembly are crucial for an analysis of the generation of antibody diversity.

Structural and genetic studies of the heavy chain can be greatly facilitated by reagents for the identification of genetic markers of the constant and variable chain segments. Allotypic specificities provide such markers: the Fd, variable portion is characterized by three specificities Al, A2, A3, controlled at the *Aa* locus by three respective alleles (4, 5). The constant, Fc portion of the gamma chain is characterized by two groups of allotypic specificities, *Ad* and *Ae* (3, 6, 7). Within each group two specificities, controlled by two allelic genes, have been described. All three loci, *Aa, Ad* and *Ae* are closely linked with each other. Recombinants have been observed, both between *Aa* and *Ad* (8) and between *Aa* and *Ae* loci (9).

So far, only limited population studies on the genetics of the *Aa* allotypes have been reported (10, 11) and even less information is available on the Fc gamma specificities (*Ad* and *Ae*). Since we were in possession of a rather unique collection of rabbit sera which represented a random-bred, panmictic and unselected population, it was decided to use this collection in the present study. We concentrated on the specificities controlled at the *Aa* and *Ae* loci and intended to answer the following questions: (a) Can the genes within each group or "locus" be regarded as true alleles? (b) Are there any unknown alleles involved in either of the two loci? (c) Are there any preferential associations between the variable and constant portion genes? In other words, is there any evidence for the presence of selective pressure acting in favor of or against some genotypes?

Materials and Methods. Rabbit sera were collected from random-bred rabbits. All rabbits came from a number of small breeders in Ontario. Our collection of sera represented a population which was very heterogeneous in its allotypic composition. All known allotypic specificities except A6 were present in our collection. The sera were stored at —15° for periods of 1–10 yr. The heterogeneity of the population in study is in a marked contrast to the allotypic composition of rabbit colonies maintained in most research institutions. These colonies usually originated from a relatively small number of parental animals and, consequently, have a very limited gene pool.

Typing. The techniques of allotype determination and the source and methods of production of the reagent antisera were described before. Determination of the *Aa* specificities was done using precipitating antisera and the double diffusion technique in agar. For the determination of the *Ae* specificities, the passive haemagglutination inhibition test described before was used (6). In this test the previously used nondisposable haemagglutination trays were replaced with disposable Mictotiter trays with "V" cups (Cooke Engineering Company, Alexandria, VA, Cat. No. 220-25).

Results. Table I gives the frequencies of

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TABLE I. Genotypes of the *Aa* Locus Found in the Sample of 1730 Outbred Individuals.[a]

Genotype	No. of individuals observed (expected)	Frequency	X^2	Gene frequency[b]
a^1/a^1	587 (568.80)	*0.3393*	0.5822	$a^1 = 0.5734$
a^1/a^2	134 (151.55)	*0.0775*	2.0319	$a^2 = 0.0763$
a^1/a^3	676 (695.11)	*0.3908*	0.5256	$a^3 = 0.3503$
a^2/a^2	12 (10.07)	*0.0069*	0.3693	
a^2/a^3	106 (92.38)	*0.0613*	2.0074	
a^3/a^3	215 (212.29)	*0.1243*	0.0346	
Total	1730		5.5510 $.25 > p > .1$ $df = 3$	

[a] The expected numbers of individuals were calculated from the gene frequencies. df = degrees of freedom. p = Probability that the differences between the observed and expected numbers are *not* statistically significant.

[b] Gene frequency was obtained by direct gene count.

the genotypes at the *Aa* locus in the population under study. The gene frequencies were calculated by direct gene count and then used to calculate the expected number of individuals of each genotype. The agreement between the observed and the expected numbers is fairly good ($.25 > p > .1$). Having thus found that the *Aa* genes in our population are in equilibrium, we proceeded to the typing of the *Ae* specificities. Table II summarizes the results of this typing.

The samples were divided into subpopulations according to their *Aa* genotypes and the frequencies of the *Ae* specificities and genes were determined in each subpopulation. Taking into account the linkage between the *Aa* and *Ae* loci, we observed the following "chromosomes" or gene pairs in our population: a^1-e^{14}, a^1-e^{15}, a^2-e^{14}, a^2-e^{15} and a^3-e^{15}. The gene pair a^3-e^{14} was not found in any of the 213 homozygous individuals. Having identified the above gene pairs, we then asked if these pairs are found in comparable frequencies in different subpopulations (see Table III). First, the frequency of the gene pair a^1-e^{14} was found for the subpopulation I; next, the frequency of e^{14} was calculated for the gene pool a^1 of the subpopulation II. This was done under the assumption that there were no a^3-e^{14} gene pairs in this subpopulation (since there were no a^3-e^{14} genes in the a^3/a^3 subpopulation); consequently all e^{14} genes should be in coupling with a^1.

In subpopulation I and II the frequencies of a^1-e^{14} were 0.070 and 0.071, respective-

TABLE II. Distribution of *Ae* Genotypes in the Five Subpopulations of Different *Aa* Genotypes.

Ae Genotype		Subpopulation: *Aa* genotype; no.: *frequency*						Total
		a^1/a^1	a^1/a^2	a^1/a^3	a^2/a^3	a^3/a^3	a^2/a^2	
e^{14}/e^{14}	No.	0	1	0	0	0	0	1
	f		*0.0075*					0.0014
e^{14}/e^{15}	No.	14	25	12	2	0	0	53
	f	*0.1400*	*0.1880*	*0.0710*	*0.0189*			0.0723
e^{15}/e^{15}	No.	86	107	157	104	213	12	679
	f	*0.8600*	*0.8045*	*0.9290*	*0.9811*	*1.0*		0.9263
Total		100	133	169	106	213	12	733
Gene frequency								
e^{14}		0.07	0.1015	0.0355	0.0094	0.0	0.0	
e^{15}		0.93	0.8985	0.9645	0.9906	1.0	1.0	

TABLE III. Frequency of the Genes e^{14} and e^{15} in the Three Gene Pools a^1, a^2 and a^3.

Subpopulation			*Ae* gene frequencies		No. of individuals observed (expected)[a]					
Designation	Genotype	Gene pool	e^{14}	e^{15}	e^{14}/e^{14}	e^{14}/e^{15}	e^{15}/e^{15}	N	χ^2	p
I	a^1/a^1	a^1	0.0700	0.9300	0 (0.50)	14 (13.10)	86 (86.40)	100	0.0637	$0.950 > p > 0.900$
II	a^1/a^3	a^1	0.0710	0.9290	0	12	157	169	0.0008	$0.990 > p > 0.975$
		a^{3b}	0	1.0	(0)	(11.91)	(157.09)			
III	a^1/a^2	a^{1a}	0.0705	0.9295	3	25	107	131	12.6877	$p < 0.005$
		a^{2c}	0.0310	0.9690	(0.29)	(12.91)	(119.79)			
IV	a^2/a^3	a^2	0.0310	0.9690	0	2	104	106	0.0162	
		a^{3b}	0	1.0	(0)	(3.29)	(102.71)			

[a] Gene frequencies a^1-e^{14} and a^1-e^{15} were 0.0705 and 0.9295 respectively, the averages from subpopulations I and II.

[b] Gene frequencies a^3-e^{14} and a^3-e^{15} were 0 and 1.0, respectively (from results in Table II).

[c] a^2-e^{14} = $f a^1$-e^{14}.

ly. A mean of these frequencies (0.0705) was assumed to be the frequency of a^1-e^{14} in the subpopulation III (a^1/a^2). The overall frequency of e^{14} in this subpopulation was 0.1015. If we assume that the frequency of the gene pair a^1-e^{14} is the same (*i.e.*, 0.0705) in all three subpopulations, then the higher frequency of e^{14} in the subpopulation III must clearly be attributed to the a^2-e^{14} gene pair (0.1015 — 0.0705 = 0.031).

The frequencies of all five gene pairs were then used to calculate the expected numbers of individuals in each subpopulation. The results of these calculations are summarized in Table III. The agreement between the observed and the expected numbers was remarkably good, except for the subpopulation III, where a statistically significant excess of double heterozygotes (a^1/a^2, e^{14}/e^{15}) was found.

Discussion. The data presented in the preceding section confirmed further the hypothesis that a^1, a^2 and a^3 are allelic genes. No phenotypes were found which would suggest the presence of an unknown allele at either *Aa* or *Ae* locus.

A remarkable finding was a complete lack of the a^3-e^{14} gene pair in the entire sample. This was directly established for the subpopulation a^3/a^3 and indirectly for the subpopulations a^1/a^3 and a^2/a^3. The total number of individuals is 486, the total numer of a^3 genes involved in 699. This phenomenon can be explained by: (a) the inability of e^{14} to express itself if in a *cis* position with a^3; (b) the relative low frequency of recombinations leading to the formation of the a^3-e^{14} gene pair; or (c) a negative selective value of a^3-e^{14} chromosome. This chromosome may, for instance carry a lethal gene at another locus, closely linked with *Aa* and *Ae*.

Oudin and Tosi (personal communication) found the a^3-e^{14} pair in several rabbit families bred at the Institut Pasteur in Paris; the typing has been confirmed in our laboratory. This observation allows us to exclude the first possibility, but not to decide between the remaining two.

Rather unexpected was the finding of an excess of double heterozygotes (a^1/a^2,

e^{14}/e^{15}) in the subpopulation II. It is difficult to speculate about the mechanisms responsible for this phenomenon. Our test does not enable us to distinguish between the two genotypes a^1-e^{14}/a^2-e^{15} and a^1-e^{15}/a^2-e^{14} and, therefore, we cannot tell whether the overall excess of double heterozygotes is due to a higher than expected frequency of both genotypes or whether this excess can be attributed to only one of the two genotypes.

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