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Review

Multiple Expression of Rabbit Allotypes: The Tip of the Iceberg?

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The discovery of rabbit allotypes (Oudin 1960) led directly to a large number of basic concepts in immunology. The study of these genetic markers of the rabbit immunoglobulins has progressed considerably (for reviews see Mage *et al.* 1974 and Kindt 1975), but recent findings raise questions which may well force the reinterpretation of many previous results and may unravel a new aspect of the transmission of regulatory mechanisms in mammals.

I. The Various Rabbit Allotypes

To familiarize the reader with the subject I will first summarize briefly the important rabbit allotypes and the role they have played in the emergence of our current ideas concerning immunoglobulins.

A. Rabbit a Allotypes

The rabbit a-group allotypes are located on the variable region of the heavy chains, and thus provide a major argument for an independent genetic control of the variable and constant regions. The Todd phenomenon, the fact that a1, a2, or a3 markers can be found on the heavy chains of any immunoglobulin class (Todd 1963, Feinstein 1963) has, after many years, found its ultimate confirmation in amino acid sequence determinations on pooled IgG and IgA variable regions (Mole *et al.* 1971, 1975, Pratt and Mole 1975, Johnstone and Mole, in preparation). The presence of the a markers in the V region has long been a major argument against the germline theory, since it was argued that an ad hoc mechanism had to be imagined to conserve the structural correlates of the *a*-locus allotypes on thousands of V-region genes. Such a conservation would have to be done for the three best known alleles, *a1, a2,* and *a3,* for the alleles found in wild rabbits, *a100, a101, a102,* and *a103* (Brézin and Cazenave 1976, Cazenave and Roland 1976) and also for the alleles of the x and y group allotypes.

B. Rabbit b Allotypes

The rabbit b-group allotypes are located on the kappa light chains. Amino acid sequence studies have established that the b allotypes (b4, b5, b6, and b9) correlate with multiple amino acid substitutions in the constant region (Strosberg *et al.* 1976, Goodfliesh 1975, Zeeuws and Strosberg 1975, Farnsworth *et al.* 1976, Strosberg and Janssens 1976) of the kappa chains. While amino acid residues have been found that are unique to the amino terminal sequences of some light chains of a given allotype (i.e., allotype-associated residues), these residues are not demonstrated in all light chains having that allotype (in this case these residues would be called allotype-specific). The existence of the allotype-associated residues in the variable region could be explained as a result of selective combination of distinct sets of variable regions with a gene for a given constant region allotype. For example, partial local inbreeding for the rare b6 or b9 allotypes could have led to the selection of some variable region genes rather than others. This inbreeding hypothesis is supported by the fact that the whole b6 population of the United States probably originated from a single b6 rabbit imported from France (Todd, personal communication).

C. Rabbit d and e Allotypes

The rabbit d- and e-group allotypes are located in the constant region of the gamma chain and differ by only a single amino acid substitution (Prahl *et al.* 1969, Appella *et al.* 1971). The importance of the d- and e-group allotypes is in their use in linkage analyses of V- and C-region genetic markers.

D. Other Allotypes

The x- and y-group allotypes, located on the heavy chain V-region, and the markers of the μ chains, will not be discussed here for lack of data. An excellent review on alpha chain markers appeared recently (Knight and Hanly 1975).

II. Expression of Rabbit Allotypes

A. Classical Concept of Rabbit a- and b-Group Allotypes.

The classical concept of the a- and b-group allotypes is that they are coded for by loci, each existing in a number of different forms or alleles. For the *a* locus there are three alleles (*a1, a2,* and *a3*) in the domestic rabbit, to which one may add *a100, a101, a102,* and *a103* if one considers the wild rabbit. For the *b* locus there are four alleles (*b4, b5, b6,* and *b9*) for the domestic rabbit. All these alleles behave in a Mendelian fashion: *a* and *b* markers are not linked but are subject to allelic exclusion, *i.e.,* in heterozygous cells only one of the two alleles present in the genome is expressed. In newborn animals,

if one suppresses the production of all forms of a allotype-bearing molecules, the production of x or y molecules is greatly enhanced. Similarly, suppression for kappa-chain allotypes of the b group results in the increased synthesis of lambda chains, which carry another type of marker controlled by the *c* locus. The c allotypes c7 and c21 were considered originally to be analogous to the b markers. Surprisingly, they are much more prevalent in b9 rabbits than in others. When c7 and c21 animals were mated, the markers were not always found to behave in a Mendelian fashion: to obtain the observed distribution of the markers in the offspring, one had to propose the existence of $c7^-$ and $c21^-$ alleles, and one had to suggest that *c7* and *c21* were not different forms at the same loci, but rather, independent loci: *c7* and *c21* behaved like pseudoalleles (Gilman-Sachs *et al.* 1969).

B. Evidence for a Regulated Expression

1. Pecking Order of the a and b Markers. Heterozygotes for both the *a* and *b* loci do not express these markers in a 50–50 proportion. Some alleles seem to be privileged, and one may define a pecking order when considering both the wild and domestic rabbit markers: a101 > a1 > a3 > a2 > a100, and b4 > b6 > b5 > b9. Different pecking orders may be defined when animals are hyperimmunized with various antigens. Several reports have described a preferred allotype on antibody molecules produced in heterozygotes. A most striking example was found in our laboratory when an a1a101b4b5 rabbit was immunized with *Micrococcus lysodeikticus*. The preimmune serum contained 70 percent a101 and 30 percent a1 molecules. Hyperimmunization led to the production of antibodies of very restricted heterogeneity, exclusively of allotype a1, b5 (Strosberg, Roland, and Cazenave, unpublished results). The pecking order may reflect different sizes of variable-region gene libraries for the a-group allotypes, different general characteristics of the variable regions (such as the overall charge), or different regulatory mechanisms. Clearly, the distinction between these possibilities is dependent upon what one believes to be the size of the immunoglobulin variable-region gene pool.

2. Allotype Suppression. Allotype suppression of a- and b-group determinants, but not of other allotypic determinants, can be induced either in vivo or in vitro (reviewed in Mage 1975, Adler 1975, Sell 1975, Catty *et al.* 1975). Allotype suppression may be maintained in vivo by a B-cell autoantibody control mechanism, as is suggested by the fact that chronically suppressed rabbits can be induced to produce antibody to the suppressed allotype, and that suppressed animals may maintain a low titer of the suppressive antiallotypic antibody for many months, too long for the antibody to be of exogenous origin, be it the initial suppressive injection or maternal transfer (Lowe *et al.* 1975). These autoantiallotype antibodies could control the T- and B-cell allotype production, and serve as a way of regulating directly the production of allotypic markers (Sell 1975, Strosberg 1976). This hypothesis could also explain the very rapid disappearance of unexpected allotypes in the animals described in section III.

It has been suggested that the suppressive autoantiallotype antibody is produced by maternally derived lymphoid cells that cross the placenta during fetal life. However, Rodkey (personal communication) describes this production in a two-year-old animal which was never experimentally suppressed. Since most of the maternal immunoglobulin becomes undetectable after six months, one must conclude that the antiallotype antibodies are of autologous origin.

3. Somatic Recombination or Regulatory Leakage? The allotypic combinations of the majority of heavy-chain molecules present in the serum of one rabbit reflect the parental linkage groups of V_H and C_H allotypes. If one parent has the a2 and d12 markers, and the other a3 and d11, the progeny molecules will bear either the a2d12 or the a3d12 combination. There appears, however, to be a small percentage of molecules that have allotypes of the recombinant type, in which genetic information for the variable region of the heavy chain is derived from the maternal chromosome and that for the constant region from the paternal chromosome, or viceversa. Several recent reviews have discussed the origin of the so-called recombinant heavy-chain molecules in which, instead of the usual cis transmission of the a, d, and e markers, one is able to detect from 0.3 to 3 or 4 percent of molecules which apparently bear markers from both the maternal and paternal chromsomes (Mage *et al.* 1974, Kindt 1975, Knight and Hanly 1975). The usual interpretation of these data involves somatic recombinational events. If, however, the a1, a2, and a3 genes are not truly allelic to each other, but are closely linked, one need not evoke somatic recombination to explain what may simply be a regulatory effect. Although the d and e markers appear to be much simpler chemically than the a and b allotypes, nothing distinguishes them from the a or b allotypes genetically. The d11 and 12 molecules may also be products of closely linked genes, and again, their expression could be regulated in an inherited fashion. What are regarded as recombinant molecules may represent low levels of the normally produced alternate combinations. Low levels of normally produced alternate products or combinations may, at early stages, induce the synthesis of the previously discussed autoantiallotypes. Anti-a-allotype antisera are very difficult to raise experimentally, which would be expected if the allotypes were common to all individuals of a species.

III. Deviations from the Classical Behavior of Allotypes

A. Expression of Unexpected Allotypes in Domestic Rabbits

Although the Mendelian behavior of rabbit a and b allotypes has been well established in many breeding studies, several recent findings are difficult to fit within this concept.

1. Discovery of a Pluriallelic a1a2a3b4b5b6 Rabbit. After hyperimmunization with lyophilized *Micrococcus lysodeikticus* bacteria, a rabbit of allotype a1a3b4b5 (rabbit 136) was found to express three alleles of the *a* group (*a1, a2* and

a3) and three alleles of the *b* group (*b4, b5,* and *b6*; Strosberg *et al.* 1974). As much as 3 mg/ml of serum carry the unexpected markers. Isolated light chains were shown to contain the b4, b5, and b6 specificities, and the heavy chains of the a2 specificity were indistinguishable from normal a2 chains by peptide mapping (Mole and Strosberg, unpublished results). Serial bleedings indicated that certain allotypic specificities were present only transiently: occasionally a2, b6, or b5 could not be detected. Recent results (Mandy and Strosberg, unpublished observations) also reveal the presence of the d11 marker.

2. Transitory Allotypes. Recent data by Mudgett and coworkers (1975) suggest that a high proportion (50 percent) of normal rabbit sera express low levels of group-a allotypes not anticipated by breeding data. Immunoglobulins possessing these latent markers are present in amounts between 0.1 and 44.0μg/ml (0.02 to 1 percent of the IgG), and are found in individual animals in a sporadic and transitory fashion.

3. Latent Allotypes. In a series of unpublished experiments, Francis and Mandy have screened pre- and postimmune bleedings of a group of 200 rabbits hyperimmunized with ovalbumin. Extremely low amounts (1 to 50 μg/ml) of a1-allotype bearing molecules were detected repeatedly in animals identified previously as homozygous or heterozygous for the a2 and a3 allotypes. In several hyperimmune animals, occasional bleedings contained 200 to 398 μg of a1 or a2 molecules per ml of serum, and these molecules could be detected by precipitin lines in agar immunodiffusion. In the case of the light chain markers, several unexpected allotypes were seen: in one family a latent b4 was recognized in a mother (b5, b6) and one sibling (b6, b6), but not in other siblings (b5, b6). Up to 620 μg/ml of b4 molecules were detected in one serum. As in the case of the pluriallelic rabbit 136, the latent allotypes were seen in a transitory and unpredictable fashion: several bleedings were negative, then one positive, then again several negative.

4. Other Pluriallelic Rabbits. Pluriallelic rabbits were also found by Wolf and his collaborators. When a nonimmunized a1a2a3 buck was mated with a homozygous a3 doe, three types of offspring were obtained: a2a3, a1a3, and a2a3 (Wolf, personal communication).

5. Unexpected Allotypes from RNA-treated Cells. Two reports by Bell and Dray (1969, 1973) suggest that lymphoid cells of one rabbit treated in vitro or in vivo with lymphoid RNA from a rabbit of different phenotype, synthesize immunoglobulin carrying light- and heavy-chain allotypes of the RNA donor. The authors interpret the continued and increased magnitude of the response after 37 days as suggesting the survival and proliferation of RNA-treated cells, rather than persistent donor-RNA directed protein synthesis. The presence of stable donor-RNA is, however, not unlikely and is supported by the results of Bilello and coworkers (1975) showing that RNA from antigenically stimulated rabbit macrophages, when introduced in a mouse cell-free system, leads to the production of rabbit Ig.

B. Expression of Unexpected Allotypes in other Species.

In mouse, rat, and human experimental systems, similar results which have been difficult to interpret in the terms of the classical allelic system of immunoglobulin markers have been reported.

1. The Mouse. Under conditions of stress, certain inbred mice express an unexpected C_4 allele (Bosma and Bosma 1974). Indeed, animals of a congenic partner strain of BALB/c mice, the ICR CB-17 strain, were specially bred so as not to differ from BALB/c mice in any known way except to carry immunoglobulin structural genes for the C57BL/Ka allotype. In certain circumstances, the BALB/c allotype appeared in the serum of some ICR CB-17 mice, previously thought to be allotypically homozygous. The appearance of this previously hidden allotype was usually transient and associated only with immunoglobulins of the IgG (IgG_{2a}) class.

2. The Rat. An experiment was designed to examine the possible expression of rat immunoglobulin allotypes by thymus-dependent cells. Radiation chimeras were constructed by injecting into thymectomized irradiated rats (allele *Ig-1*^{b}) thymus-dependent cells carrying the rat kappa light chain allotype (allele *Ig-1*^{a}). After immunization with sheep erythrocytes or dinitrophenylated bovine gamma globulin, a direct binding assay was used to determine the percentage of Ig-1^a or Ig-1^b allotype in whole antibody. In 24 out of 26 animals there was no detectable allotype derived from the thymus cell inoculum. However, one animal showed about 10 percent and another about 20 percent of the thymocyte-specified allotype. It is hard to explain the anomalous findings in these two animals by a contamination of the inoculum with thymus-dependent cells and one must consider one of the following two alternative explanations: either thymus-dependent cells can influence the allotype of the eventual antibody or the normally repressed *Ig-1*^{a} allele can be expressed in *Ig-1*^{b} animals (Hunt and Duvall 1976).

3. Man. Lobb and coworkers (1967) detected an apparent discrepancy between the genotype and the expressed phenotype of an individual after the cells were grown in culture. This conclusion was drawn from their finding that both Gm(a) and Gm(b) molecules could be detected by immunofluorescence on cells from a^+b^- or a^-b^+ donors. Rivat and coworkers (1970, 1973) also suggest that human lymphocytes may express unexpected C_H allotypes when cultured in vitro with allogeneic cells in a mixed lymphocyte culture. In a human lymphoid cell line transplanted in neonatal Syrian hamsters, Pothier and coworkers (1974) detected an intermittent expression of Gm(a), even though this marker was not present in the donor phenotype of the serum.

C. Deviation from the Classical Behavior of Histocompatibility Antigens.

Histocompatibility antigens constitute another system in which a large number of proteins appear to be products of allelic genes, but in which certain anomalies

have prompted Bodmer (1973) to question the classical Mendelian concept. Results by Garrido and coworkers (1976) indicate that when a chemically induced murine tumor cell line was infected by vaccinia virus, the expression of H-2 specificities from haplotypes other than the one of the original mouse strain was observed.

IV. Structural Correlates of Rabbit Allotypes

A. Definition

The structural correlates of rabbit allotypes have been defined by using the following syllogism: two immunoglobulins behave differently with monospecific antiallotype antisera: they thus bear different genetic markers. Since they also differ consistently at a given location in, say, position 3, this amino acid substitution correlates with the difference in allotypes: it *is* the structural correlate of the considered allotype. The best documented structural correlate of a rabbit allotype is the d11, d12 difference. (Prahl *et al.* 1969). McBurnette and Mandy (1975) isolated and sequenced the d11 peptide and showed that it inhibits the d11 anti-d11 reaction. Since, in many systems, several markers correspond to single amino acid substitutions, it has been accepted widely that allelic genes should differ only by a few residues. In the situations in which this is true, allotype anti-allotype reactions are quite difficult to detect, and rarely result in immunoprecipitations. For example, the rabbit *d* and *e* markers, like most human Gm markers, can be characterized only by hemagglutination or binding assays. The a and b markers, however, are characterized by multiple amino acid substitutions, and they react with their respective antibodies by yielding immunoprecipitates. Single (or a few) substitutions thus probably generate a single antigenic determinant, and antibody-antigen complexes formed with antisera against this determinant remain soluble, since they can at best form two dimensional lattices because of divalent antibody molecules. Multiple substitutions, on the other hand, generate at least three antigenic determinants, sufficient to lead to the formation of precipitating three dimensional complexes.

B. The a Group Allotypes

Examination of the amino acid sequence of the V_H region from normal pooled rabbit IgG has revealed segments different for a1, a2, or a3 (Wilkinson 1969, Mole *et al.* 1971). Data from homogeneous antibodies have confirmed the presence of allotype-specific or allotype-associated residues at positions 4–16, 63–73, and 84–85 (Fig. 1; Jaton and Braun 1972, Jaton *et al.* 1973, Strosberg *et al.* 1972a, b, Fleischman 1973). For the 24 positions compared, allotype a1 and a3 H chains differ by five to seven residues, and a2 chains differ from either a1 or a3 by twelve to fifteen residues. The multiple-sequence differences between allelic forms of the a-group allotypes could not have all appeared at the same time. Although mechanisms for the appearance of substitutions between duplicated genes have been described, they are difficult to test. If

	4	7	9*	11	12	14	15*	16*	28	64	66	69	70	73	76	83	84
a_1	E	G	R	V	T	G	T	P	L	G	F	S	K	T	D	T	E Q
a_2	K	E	G	F	K	T	B	T	L	S	S	T	R	B	B	A	Q
a_3	E	G	D V	V	K	G	A	S	G A	G	F	S	K	T	E	A	A

Fig. 1. Allotype-specific(*) and allotype-associated residues in the variable region of a1, a2, and a3 rabbit heavy chains. Compounded sequence data for a1 and a3 normal pooled H chains are from Wilkinson 1969, Mole *et al.* 1971, and Johnstone and Mole (manuscript in preparation). Sequence data for homogeneous H chains are from Jaton and Braun 1972, Fleischman 1973, Strosberg *et al.* 1972b and Van Hoegaerden and Strosberg 1976

the appearance is progressive, one would expect the existence of intermediate forms. Serological subspecificities have been reported for a1, a2, and a3 molecules (Oudin 1960, Brézin and Cazenave 1976, Horng *et al.* 1976) and it is tempting to suggest that these may correspond to the intermediate forms. An a1 molecule with the gly-gly-ser sequence (Cannon *et al.* 1976) is closer to the a3 gly-ala-ser sequence than to the gly-thr-pro sequence identified in most other a1 molecules reported (Van Hoegaerden and Strosberg 1976, Johnstone and Mole, in preparation). The existence of the variable-region a allotypes constituted one of the major arguments against the germline theory of antibody diversity, since it was argued that a number of V genes of each allotype would have led to numerous recombinations. It is now generally accepted that multiple variants coexist on the same chromosome in all animals for each a allotype (Brézin and Cazenave 1976). Nothing prevents allelic forms of genes at the same locus from differing by many substitutions. However, if heterozygotes gain a selective advantage over homozygotes, evolutionary pressure could possibly favor gene duplication which would create homozygotes with both alleles coexisting on the same chromosome. This would confer polymorphism on individuals rather than on the species. The close linkage would appear as pseudoallelism in genetic studies.

C. The b-group Allotypes

Recent results obtained from this and other laboratories indicate that the constant regions of kappa light chains of allotype b4, b6, and b9 differ by several amino acid substitutions (Zeeuws and Strosberg 1975, Goodfliesh 1975, Chen *et al.* 1974, Janssens and Strosberg 1976, Strosberg *et al.* 1972a, Farnsworth *et al.* 1976). A comparison of all available sequences is presented in Figure 2. The b4 and b6 constant regions differ by 15 residues out of 54 analyzed, b4 and b9 by 33 residues out of 103 sequenced, and b6 and b9 by 17 out of 54 analyzed. These extensive differences contrast with the dramatic similarity between b4 and b6 variable-region partial sequences (Strosberg *et al.* 1976), and suggest an independent evolution for V and C genes. Because of the large number of amino acid substitutions, the diversification of constant regions into

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      110                 120                 130                 140
b4  D P V[ ]A P T V L I F P P A A D Q V A T G T V T I V C V A N K Y F P[ ]D V T V T W E
b6        G A P T V L L F P P ? ? S E L A T G T A T I V C V A B K Y F P[ ]D T G V T W K
b9  D P P I A P T V L L F P P S A D Q L T G Z T V T I V C V A N K F R P D D I T V T W K
b5                                                  I V C V A N K

      150                 160                 170                 180
b4  V D G T T Q T T G T Q D S K T P Q D S A D C T Y N L S S T L T L T S T Q Y N S
b6                              T P Q D G S G C T
b9  V D D E I Q Q S G I E N S T T P Q S P E D C T Y ? L S S T L S L T K A Q Y N S
b5                              T P Q N S D D C T

      190                 200
b4  H K E Y T C K V T Q G T T S V V Q S F N R G D C
b6                                    S R K S C
b9  H S[ ]Y T C Q V H N S A G S I V Z S F N R G N C
b5        E Y T C K                   S R K D C
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Fig. 2. Allotype-specific and allotype-associated residues in the constant region of b4, b5, b6, and b9 rabbit light chains. Compounded sequence data are from Strosberg *et al.* 1972a, b, Chen *et al.* 1974, Frangione 1970, Appella *et al.* 1969, Goodfliesh 1975, Farnsworth *et al.* 1976, Zeeuws and Strosberg 1975, and Strosberg and Janssens 1976. Empty brackets [ ] indicate deletions introduced to maximize homology

several polymorphic forms obviously must have occurred early in evolution. Gene duplication probably took place before speciation in the lagomorphs, a theory supported by the existence of b4, b5, b6, and b9 antigenic determinants in hares (Landucci-Tosi *et al.* 1973, Cazenave and Roland 1976) and by polymorphism among hares of various origin (Cazenave and Roland 1976, van der Loo *et al.* 1976, Mandy, personal communication). In light of the existence of simple allotypes (Gutman *et al.* 1975), in which alleles differ only by one or a few residues (d11 and d12 or e14 and e15) one may again wonder whether both the a and b polymorphic forms differing by many substitutions, coexist on a single chromosome instead of being distributed in different individuals. The 24 to 30 percent substitutions among constant regions of allotypes b4, b6, and b9 must have been fixed in evolution. If a normal rate of mutation is assumed, this diversification must have taken place before the divergence of rabbit and hare, since preliminary studies suggest the presence in the hare of b6, and, to a lesser extent, of b4 and b5 determinants. The fixation of the mutations must have occurred because of distinct advantages conferred by the preservation of the extensive differences among the various constant regions. The substitutions are too important to be considered neutral and the presence of three insertions supports this conclusion. Although few studies of constant region sequences have been done, it does not appear that multiple sequence variants exist, at least for the b allotypes. The varicus b4 or

b9 light chains prepared from pools of normal immunoglobulins (Strosberg *et al.* 1972a, Farnsworth *et al.* 1976) do not differ by more than one or two residues from chains obtained from homogeneous antibodies (Appella *et al.* 1974, Chen *et al.* 1974, Margolies *et al.* 1975, Zeeuws and Strosberg 1975). Sequence variants of b4 constant regions have been reported. At position 174, three amino acids val, leu and asn have been identified. Whether several b4 sequences may be recognized in single animals is not known. In a recent study, Sogn and Kindt (1976) described the inherited appearance of a ser-ala-asp-leu sequence between positions 121 and 124 instead of the more common ala-ala-asp-gln sequence. Serological distinction of these two b4 forms was not reported and it remains unclear whether the *b4* variant gene is allelic or pseudoallelic with respect to the *b4* gene. Serological distinction of two b4 types of chains (b4–1 and b4–2) was accomplished by van der Loo and coworkers (1975). Again, the breeding studies revealed the inherited appearance of the b4–2 chains. Sequence studies (Strosberg and van der Loo, unpublished data) indicate that b4–2 is not the same as the b4 variant from Sogn and Kindt (1976), since the normal ala-ala-asp-gln sequence was found between positions 121 and 124.

V. Structural Correlates of Other Antigenic Systems in which Multiple Amino Acid Substitutions Characterize Allelic Forms

A. The Rat Light Chain Allotypes

Two serologically detectable forms have been recognized among immunoglobulin kappa chains from various inbred rat strains which segregate in a Mendelian fashion and have been designated allotypes a and b of the *RI-1* locus. Sequence comparisons at 81 positions of the constant region have shown ten amino acid substitutions and one deletion between these two forms of the kappa allotype. (Starace and Quérinjean 1975, Gutman *et al.* 1975). In addition, two sequence differences were found between the C-kappa regions of two preparations (pooled LEW chains and myeloma LOU chains) otherwise identical at the *RI-1* locus by serological analysis.

B. Histocompatibility Antigens

Recent sequence results indicate that differences among the various allelic forms of histocompatibility gene products are extensive and seem to confirm the earlier conclusions based on peptide mapping. In the human, three alleles of the *HLA* complex were examined by Terhorst and coworkers (1976) and two differences were found in the 25 positions analyzed. Four different groups confirmed, at the amino acid sequence level, the known polymorphism of the murine *H-2* complex (Ewenstein *et al.* 1976, Henning *et al.* 1976, Silver and Hood 1976, Vitetta *et al.* 1976). In the most recent study (Capra *et al.* 1976), the products of the presumably allelic genes $H\text{-}2K^b$ and $H\text{-}2K^k$ differ by at least six amino acids in the 27 aminoterminal positions. These results, together with

those of Garrido and coworkers (1976), support the original suggestion by Bodmer (1973) that the *H-2* haplotypes may represent different, closely linked genes present in each animal, and that the basis for the genetic polymorphism is in control of the gene expression. In summary, evidence for an inherited regulatory control of the expression of rabbit allotypes may be deduced from the following considerations:

1. the existence of a *quantitative regulation,* suggested by the unequal expression of allelic forms in heterozygotes (pecking order), and of a *qualitative regulation,* suggested by suppression experiments in homozygotes, leading to the expression of otherwise undetected markers, such as the x and y heavy-chain allotypes, borne by a-blank molecules.

2. the appearance of rabbits which display *more than two alleles* of the *a* and *b* loci, and of animals in which molecules are detected with allotypes not expected from the parental genotype. These latent, unexpected, or previously hidden markers have now been observed in humans, mice, and rats.

3. the high frequency of so-called *recombinant molecules,* displaying the a and d or e markers in *trans* rather than in *cis* configuration. These recombinant molecules may represent evidence of a regulatory control over the *cis* or *trans* expression.

4. the existence of *multiple differences* among amino acid sequences of molecules bearing allotypes of the a or b group.

5. the expression of various *allotypic subspecificities* or sequence variants in single animals, which at the very least suggests the coexistence on the same chromosome of several forms of a given allele.

In view of these considerations, it is tempting to propose new models to account for the Mendelian behavior of rabbit a and b allotypes, or even of all immunoglobulin markers, and for their unexpected properties in terms of their structural correlates or unexpected appearance. One such model would be that rabbit allotypes are coded for by structural genes coexisting on the same chromosome in all animals, the allelism residing in regulatory genes controlling their expression. This model is analogous to that proposed for the histocompatibility antigens by Bodmer (1973). Evidence for regulatory genes is difficult to obtain. It is possible that in a given situation (e.g., hyperimmunization, infection, or in vitro cell culture) regulation is disturbed, allowing the expression of repressed structural genes. Regulatory genes would not only have to control expression of structural genes in an independent way, but would have to account also for the existence of haplotypes, that is the inherited linked expression of certain markers.

An alternative explanation for the phenomena can be derived from the fact that rabbits may resorb their fetuses and that this resorption may result in long-term survival of chimera cells and give rise to the occasional expression of a wrong allotype in adult female rabbits. This certainly does not account

for all latent allotypes, since male animals as well as virgin females have been found to express unexpected markers. In these cases, fusion may occur between fetuses at very early stages of embryogenesis, resulting in a different form of chimerism.

In discussing the facts one should distinguish two problems: the presence in an individual of more than two alleles of a given allotype, and the regulation of their expression. Additional alleles may be always present in all animals and may appear because of a rare unequal crossingover or because of the existence of cells originating from either the mother or other fetuses. The problem of the regulation of the allotype expression is not so much the quantitative or qualitative regulation, both of which are well-established by the existence of a pecking order and suppression effects on relative levels of allotypes, but the question of an inherited regulatory mechanism which confers a Mendelian behavior on allotypes. Other systems have been studied in which given alleles appear in certain generations but not in others (reviewed by Bodmer 1973). To those one may add the inherited idiotypes in certain mouse strains (Eichmann 1975, Kuettner *et al.* 1972). Inherited idiotypes appear only during immunization of certain mouse strains with certain antigens, but will be expressed in several generations, provided immunization is continued. The distinction between idiotypic markers, probably corresponding to antigenic determinants of the hypervariable regions (Capra and Kehoe 1976), and allotype markers, especially the variable-region a allotypes, becomes more difficult. Rabbits may be induced to form autoantiidiotype antibodies, as well as autoantiallotype proteins. They may yet provide the experimental choice system for allowing the emergence of a considerably simplified picture of the regulatory control of antibody expression.

It is quite likely that the spectrum of antibody specificities expressed during the life time of a lymphocyte is considerably narrower than the total number of variable region genes present in the genome of this cell. The study of rabbit allotypes suggests that regulatory genes may restrict the expression of the genome to only a part of the repertoire and impose this restriction over all the cells of a given individual.

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