Immunogenetics 4:401–435, 1977

Immunogenetics

Meeting Report and Summary

Antibody Variable-Region Genetics:

Summary and Abstracts of the Homogeneous Immunoglobulin Workshop VII*

Martin Weigert[1] and Michael Potter[2]

[1] The Institute for Cancer Research, Philadelphia, Pennsylvania 19111
[2] National Cancer Institute, Bethesda, Maryland 20014

The emphasis of this year's workshop was on variable-region markers (V_H and V_L) with respect to new V markers, the linkage and order of V and C genes, and the structural correlates of V-region markers. This summary attempts to tabulate the data described in the abstracts and to bring up to date the most recent review of this area, *Genetic Control of Antibody Specificity in the Mouse* (K. Eichmann, *Immunogenetics 2*:491–506, (1975)). From the comparisons of the data presented at this workshop certain exciting new interpretations of the organization and evolution of the heavy-chain locus follow. We hope that this summary will in no way detract from the authors' own discussions.

V_H Gene Markers

As is shown in Table 1, the majority of V_H markers are characterized by idiotype. More recently, V-region markers have been identified using antibody fine-specificity or the isoelectric point (spectrotype) of specific antibodies or antibody L chains. Of the V_H markers identified by idiotype, several appear to be associated with a variety of sequences, and would therefore seem to be under multigene control. We will refer to these examples as Idx markers, and to the cases that appear to be specific for a single amino acid sequence as Idi markers. This nomenclature was originally proposed for the two types of antigenic determinant found among a group of functionally related myeloma antibodies (Lieberman *et al.* 1975). It should be noted that the Idx type is a broad category that would include, at one extreme, the rabbit a allotypes that occur in most, but not all, V_H regions, and at the other extreme, such closely related antibodies as the group of levan-binding myeloma antibodies or phosphorylcholine binding myeloma antibodies. Furthermore, the location of the idiotypic determinants can vary. Certain Idx determinants may result from homology between anti-

* April 1–3, 1976, Bethesda, Maryland. Sponsored by the Tumor Immunology Program of the National Cancer Institute.

Table 1. Mouse V_H Markers

V_H Marker	Assay and Reference	Antibody or Myeloma Protein	Reference Strain
DEX[a]	idiotype	– J558 MOPC104E	BALB/c
A5A^{+}[b]	idiotype, spectrotype	– induced anti-group A carbohydrate	A/J
A5A^{Cr}[c]	idiotype	– induced anti-group A carbohydrate	C57L
ARS[d]	idiotype, sequence	– induced anti-p-azophenyl arsonate	A/J
T15[e]	idiotype	– T15	BALB/c
Nase[f]	idiotype	– induced anti-staphylococcal nuclease	A/J – 1 SJL – 2
S117^{+}[c]	idiotype	– S117	BALB/c
S117^{Cr}[c]	idiotype	– S117	DBA/2, C57L
InuIdx[g]	idiotype	– E109, A47, U61	BALB/c
U10-173[h]	idiotype	– U10-MOPC173	BALB/c
NP[i]	fine specificity and spectrotype (N_1)	– induced anti-NP	C57BL/6
NBrP[j]	fine specificity	– induced anti-NBrP	C57BL/6 – BALB/c +
ABA-HOP[k]	fine specificity	– induced anti-ABA-HOP	C3H, CBA – I C57BL/6 – II A/J – III
ESE[l]	fine specificity	– induced anti-SRBC	C57BL/6 – discriminator DBA/2, BALB/c, C3H – nondiscriminator

[a] Riblet *et al.* 1975
[b] Eichmann and Berek 1973
[c] Berek *et al.* Eur. J. Immunol. in press
[d] Pawlak *et al.* 1973
[e] Lieberman *et al.* 1974
[f] Pisetsky *et al.*, these abstracts
[g] Lieberman *et al.*, these abstracts
[h] Bosma *et al.*, these abstracts
[i] Imanishi and Mäkelä 1974
[j] Imanishi and Mäkelä 1975
[k] Mäkelä *et al.* 1976
[l] McCarthy and Dutton 1975

bodies in framework regions and can, therefore, be shared by antibodies with different specificities.

Several examples of antigenic markers that occur on different BALB/c myeloma proteins have been identified. The U10-173 Idx determinant (Bosma *et al.*, these abstracts) occurs on myeloma proteins known to have different specificities (U10 binds 2,6 levan; M173 does not), and thus must result from V-region framework homologies. This determinant can also be detected on myeloma proteins that bind β1,6 galactan as well as on proteins without known specificities. Substantial amounts of this ubiquitous idiotype can be found in the normal serum (including induced antilevan antibody) of a variety of strains. The concordance of the U10-173 idiotype (C57L is U10-173-positive; AKR

is U10-173-negative) and the Ig-1a allotype of C57L in the AKXL recombinant-inbred (RI) lines shows that the U10-173 gene(s) are linked to C_H. Another example of an Idx determinant has been found on two phosphorylcholine-binding myeloma proteins, T15 and M511. These share a common antigen (or antigens) in spite of V_H and V_L differences (Lieberman and Potter, these abstracts). Thus, in distinction to the T15 determinant previously described (Potter and Lieberman 1970), unique to myeloma proteins with equivalent or nearly equivalent sequences (S107, S63, H8, and T15) and of the Idi type, this T15/M511 Idx determinant may be associated with V-region framework.

As described by Lieberman and coworkers (1975), both Idx and Idi determinants have been found on the BALB/c antilevan myeloma antibodies. The Idx determinant InuIdx is found on some but not all examples with the U10-173 Idx. Thus, the U10-173 and InuIdx determinants are different, the former related to a homology region shared by a broad group of antibodies, the latter by a region common to cases with related specificity. The InuIdx can be detected after immunization with *Aerobacter levanicum* in strains of diverse allotype. Certain strains do not express InuIdx; thus, the appropriate backcross analysis could be carried out with the result that this marker was shown to be linked to allotype (Lieberman *et al.*, these abstracts).

The V_H-Dex system has now been shown to be somewhat more complex than was previously believed. In one respect this idiotype is of the Idx type: The J558 idiotype can be detected on essentially all anti-α1,3 dextran antibody of certain strains, and is known to be related to the combining site, as based on hapten competition studies of the binding of anti-J558 idiotype to J558. A J558-like idiotype is detectable on another anti-α1,3 dextran myeloma protein, MOPC 104E, which has a somewhat different combining site (Lundblad *et al.* 1972, Carson and Weigert 1973). Though the partial V_H sequences of these myelomas show close homology (Fig. 1), since several framework differences occur in these examples (Hood *et al., Cold Spring Harbor Symp. Quant. Biol.* in press), J558 and MOPC 104E V_H regions must be coded for by separate V_H genes. The normal population of anti-α1,3 dextran antibodies has now been dissected into several species, some resembling either MOPC 104E or J558, and others distinct from these two myeloma proteins. Hence the normal antibody response to dextran consists of a number of related V_H regions. Whereas Idi types can be revealed by highly specific antisera and isoelectric focusing (Hansburg *et al.*, these abstracts; Hansburg *et al. J. Immunol.*, in press), the idiotype assayed for in strain surveys (Table 2) and in the genetic analysis of V_H-Dex (Riblet and Weigert, these abstracts) has been of the Idx type. Therefore, the V_H-Dex locus must be controlled by several V_H genes that code for similar structures differing slightly in both framework and hypervariable regions.

The idiotypes of the mouse anti-group A streptococcal carbohydrate (A-CHO) antibodies have also been shown to be complex (Berek *et al.*, these abstracts; Berek *et al., J. Exp. Med.*, in press). Part of the antibody to the A-CHO of several mouse strains has an idiotypic determinant that crossreacts with the anti-N-acetyl-glucosamine BALB/c myeloma protein S117. The induced $S117^+$ antibody of BALB/c is, however, not identical to the S117 myeloma antibody, and it is possible that the $S117^+$ portion of induced antibody is

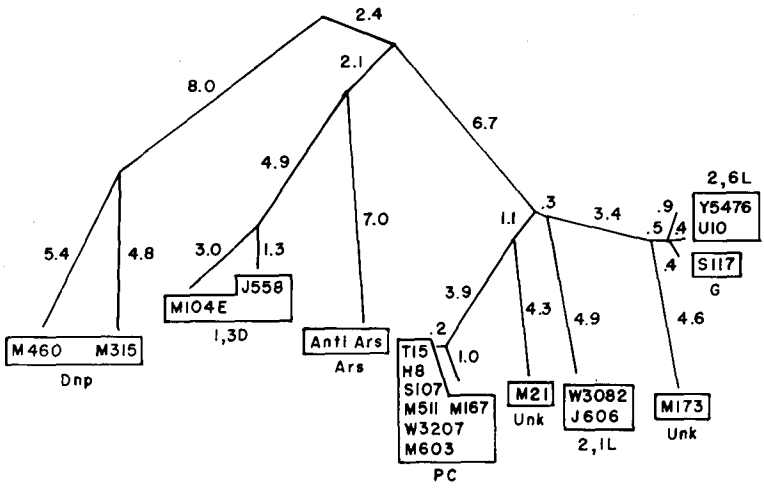

Fig. 1. A genealogical tree generated from the N-terminal 38 residues of heavy chains from 17 BALB/c proteins. Individual immunoglobulin chains are represented by number designations at the terminal twigs of the tree. The number of single base substitutions separating two sequences is indicated by numbers in the center of the line joining two corresponding branch points on the tree. (Hood *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* in press).

heterogeneous. Other strains, such as DBA/2, produce anti-A-CHO antibodies that crossreact weakly with S117 ($S117^{Cr}$ type), and these antibodies may also be a component of the $S117^{+}$-type response. This relationship is analogous to the $A5A^{+}$ and $A5A^{Cr}$ types of response to A-CHO found in other strains. In none of these cases has it been clearly demonstrated that the idiotypes are of the Idi or Idx type. In view of the homogeneity of the $A5A^{+}$ antibody demonstrated electrophoretically (Eichmann 1972) and by partial sequence analysis (Capra *et al.*, these abstracts), A5A may be a true Idi type.

The striking conclusion from these various studies is that certain V_H markers defined by idiotype are in fact found on a heterogeneous c collection of related antibodies. Though this heterogeneity could be due in part to somatic diversification, the framework heterogeneity implies multigene control. On the other hand, V_H markers such as $A5A^{+}$, and other cases defined by fine-specificity, spectrotype, and partial amino acid sequence, such as V_H-NP and V_H-ARS, may be true Idi types and under the control of single V_H genes.

Several new V_H markers were described at this workshop.

V_H-Nase (Pisetsky *et al.*, these abstracts). Antibodies to mouse anti-staphylococcal nuclease have been produced in rats that can distinguish the antinuclease antibodies of different strains, for example, SJL and A/J. These appear not to be allelic forms of a V_H gene, since BALB/c expresses antinuclease antibodies with both the A/J and SJL idiotypes. The BALB/c V_H genes in turn appear to have been separated because of intra-V_H recombination in the congenic strain BAB/14, which expresses only the A/J type. Thus two separate V_H loci express

Table 2. Strain Distribution of Genetic Markers[a]

Strain	Ig-1[b]	AKR-F_{ab}[c]	T15	DEX	S117^{+}	S117^{Cr}	A5A^{+}	A5A^{Cr}	ARS	NBrP	NP	ABA-HOP	ESE	Nase-1	Nase-2	InuIdx	U10-173
BALB/c	a	+	+	+	+		−	−	−	+	−	II	N	+	+	+	+
129	a	+	+	+	+		−	−	−								+
C58	a	+	+	+	+		−	−			−		D			+	+
C57L	a	+	+	+	+			+		+(?)	−					+	+
ST	a		+	+						+(?)	−						
IAH	a									+(?)	−						
CBA	a	−	−	−	−	−	−	−	−		−	I	D			+	−
C3H	a	−	−	−	−	−	−	−	−		−	I	N			+	−
C57Br	a			+					−				D				+
MA	a			+													
A/J	e	+		−	−	−	+		+	+(?)		III	N	+	−		+
A/He	e		−	−			+		+				N			+	
A/WySn	e								+								
NZB	e	+	−	−					−				D				+
AL/N	d		−	−	−	−	−	−	+							+	+
AKR	d	+	−	−	−	−	−	−	−	−	−		D			−	−
C57BL/6	b	−	−	−	−	−	−	−	−	−	+	II	D			−	+
C57BL/10	b		−	−			−	−			+		D			−	
C57BL/Ka	b			−						−	+						
C57BL/Ks	b									−	+		D				
SJL	b	−	−	−			−	−	−	−		II	D	−	+		+
LP	b	−							−	−	+		D				+
SM	b								−								
DBA/1	c					+	−						N				
DBA/2	c		−	−		+		+	−	+(?)	−		N			−	
RF	c	−				+		+	−	+(?)						+	+
SWR	c	−					−		−								
CE	f		−				−		−	+(?)	−		N			−	+

[a] References are given in Table 1 and in the text. + and − designations indicate the presence or absence of a particular idiotype or distinctive feature of a response such as heteroclitic antibody.

[b] Herzenberg *et al.* 1968, Potter and Lieberman 1967 [c] Spring and Nisonoff 1974

antinuclease antibodies as in A/J or SJL, or both are expressed in one haplotype, as in BALB/c.

V_H-ESE (McCarthy and Dutton 1975, McCarthy *et al.*, these abstracts). Certain mouse strains (D strains, Table 2) discriminate between types of sheep erythrocyte. This difference in the fine-specificity of response between strains has previously been shown to be allotype-linked, based on studies in congenic and RI strains. As described in these abstracts, strain surveys for this V_H marker have now been extended to the BXH and BXD RI lines.

ABA-HOP (Mäkelä *et al.* 1976, Mäkelä *et al.*, these abstracts). A V_H marker has been identified by methods analogous to those used to find V_H-NP and V_H-NBrP (Imanishi and Mäkelä 1974, 1975). The antibodies to ABA-HOP from various strains are compared for their affinity for a series of haptens relative to ABS-HOP (fine-specificity). Strain differences in fine-specificity (Table 2) have been shown to be linked to C_H.

L-Chain Markers in the Mouse

L-chain markers have been well-characterized in several species and have been defined as either simple or complex allotypes (Gutman *et al.* 1975). The human C_κ allotypes of the InV group fall in the simple category since they differ by a limited number of amino acid substitutions (Steinberg *et al.* 1974). Rabbit C_κ allotypes b_4 and b_9 (Farnsworth *et al.* 1976), or rat C_κ allotypes a and b (Gutman *et al.* 1975), are in the complex category since they differ from each other by multiple amino acid substitutions. [A similar distinction can also be made for L-chain isotypes – for example, the C_λ OZ group in the human (Ein 1968) or C_κ (LEW) versus C_κ (LOU) in the rat (Gutman *et al.* 1975) – that differ by a limited number of substitutions, as compared to $C_{\lambda 1}$ and $C_{\lambda 2}$ in the mouse that have multiple amino acid differences.] Neither kind of allotype has so far been found for either C_λ or C_κ in the mouse, though comparisons of myeloma C_κ and C_λ from BALB/c and NZB mice (Loh, E., Riblet, R., Weigert, M., and Hood, L., unpublished data) exclude complex differences between these strains.

The inability to identify mouse C_κ or C_λ allotypic determinants has led several investigators to ingenious devices that have revealed κ-chain polymorphisms between strains. The three examples described in these abstracts all appear to be located in V_κ regions. For example, strains AKR, C58, RF, and PL show the presence of a tryptic peptide, I_B, in digests of their κ chain. Because the sequence homology of the I_B peptide(s) is close to the region around CYS_{23} of certain κ subgroups, and since the I_B peptide is derived from only five percent of the total κ chain, it must be coded for by a V_κ gene (or genes) unique to these strains (Gottlieb, these abstracts, Edelman and Gottlieb 1970).

Claflin (these abstracts) has utilized the fact that all mouse strains respond to R36A pneumococci with an idiotypically restricted antiphosphorylcholine (PC) antibody. The PC response, as defined by a combining site-specific anti-H8

antiserum (H8 is a BALB/c anti-PC myeloma protein), is the same in all strains, and hence all strains have V_H and V_κ genes coding for this idiotype (Claflin and Davie 1974). The possibility, however, that strain-specific V_κ-region differences that do not influence H8 idiotype might occur in H8-associated κ chains was explored by taking advantage of the homogeneity of these L chains. For example, isoelectric focusing of H8-like anti-PC antibody L chains of C57L and AKR showed restricted L-chain bands with strain-specific isoelectric points. The isoelectric point of this L chain, $\kappa_{PC}8$, for C57L and other strains is designated $\kappa_{PC}8B$, and for AKR, C58, RF, and PL, $\kappa_{PC}8A$. Since this type of difference has so far been observed only for antibodies that share the H8 idiotype, this κ-chain polymorphism is probably located in the V-region framework of a particular V_κ subgroup. Other κ-chain differences between strains can also be detected by comparison of the spectrum of bands obtained by isoelectrofocusing of the entire L-chain pool (Gibson *et al.*, these abstracts, Gibson 1976). Fully reduced and alkylated L chains show a reproducible pattern of about 50 bands. Comparisons of these patterns between strains such as AKR and C57L show band differences with respect to the presence or absence of particular bands. Since these differences (designated IF-I, -II, etc.) comprise only from two to five percent of the total κ-chain population, the differences are again located in V_κ regions.

These recent cases of V_κ differences, as had been shown previously for the I_B peptide (Edelman and Gottlieb 1970), are found between the set of high leukemic strains AKR, RF, C58, and PL, and all other strains tested, including C57L. Utilizing the Taylor AKXL RI lines, linkage of both the $\kappa_{PC}8$ and the IF-type V_κ markers to Ly-2,3 on chromosome 6 has been established as had been shown previously for I_B (Gottlieb 1974). Though it is conceivable that all these approaches have identified differences in the same V_κ gene product, it is more likely that each approach is revealing alleles of different V_κ subgroups. For example, the I_B peptide is present on five percent of V_κ. IF differences can be seen so far on from two to five percent of the total κ chain, and the $\kappa_{PC}8$ is clearly a distinct subgroup. Thus it seems that the V_κ locus in the mouse is analogous to mouse V_H in being comprised of many loci (at least as many as the number of V_κ subgroups).

Two approaches have been adopted to estimate the minimum number of V_κ subgroups and thereby the minimum number of V_κ genes. As described in these abstracts (Loh *et al.*), sequences of V_κ regions from BALB/c and NZB are remarkably heterogeneous. Using framework differences as a definition of subgroups, at least 15 NZB and 37 BALB/c subgroups have been found from partial sequences. On the basis of the repeat frequence of certain subgroups, one can predict that at least from 50 to 100 κ subgroups will be found in an inbred strain (Cohn *et al.* 1974). This approximation is supported by the proportion of κ chains with a particular V_κ marker. Since different subgroups are coded for by different V_κ genes, a V_κ-region allotypic difference that occurs between subgroups might be found on only one to two percent of the total V_κ. The levels of the V_κ allotypes reported here are consistent with this prediction, *i.e.*, five percent for I_B, or two to five percent for AKR-specific IF bands.

Another estimate of the number of V_κ subgroups comes from the patterns obtained by isoelectrofocusing of the total κ chain of an inbred strain. The type of banding pattern given by a collection of L chains within a subgroup has been shown to be simple (Geckeler *et al.*, these abstracts). Normal mouse λ appears as a single IF band with an isoelectric point equivalent to $V_{\lambda 0}$-type myeloma L chains (the sequence found for about 60 percent of the myeloma λ chains). Variants of the V_λ subgroup with unique isoelectric points will each represent a minor proportion of the total λ population, and would not be detected by the radioautographic methods used to detect bands. Operationally, therefore, members of a subgroup will appear as single bands. By this interpretation, each band observed by Gibson (these abstracts) for κ chain constitutes the majority of L chains of a given κ subgroup. The number of bands, therefore, provides a lower estimate of the number of subgroups of V_κ (ca. 50), a number in agreement with the estimates from V_κ chain sequences. This value provides a minimum estimate of the number of V_κ genes, though each subgroup could, in turn, be coded for by a large number of similar V_κ genes. However, the finding of allotypic differences between IF bands suggests that this is not the case. If an IF band shown to be unique to a given strain were coded for by multiple genes, the same kind of mutation that alters the mobility of the band in a different strain would have had to occur in each of these genes. Hence the relationship, one band:one subgroup:one gene, seems more reasonable.

Structural Correlations of V_H Markers

Structures of mouse V_H regions have been studied extensively. Until recently, most analyses were carried out on BALB/c myeloma antibodies and were concentrated on the aminoterminal framework region (Barstad *et al.* 1974). Now a number of V_H sequences of mouse myeloma antibodies have been completed (Rudikoff *et al.*, Vrana *et al.*, these abstracts) and partial sequences of several normal induced antibodies are available (Capra *et al.* 1975, these abstracts). Comparisons of the aminoterminal V_H sequences show a remarkable degree of heterogeneity in this region of the framework. Again, by the criterion that each unique framework sequence defines a V-region subgroup and that each subgroup is coded for by a different V gene, the V_H locus must consist of a large number of genes. It can be estimated from the minimum number of V_H subgroups (Cohn *et al.* 1974) that there are at least 100 V_H genes for BALB/c, and since this estimate is based on only the examples of V_H with free aminotermini (found on less than 25 percent of the normal V_H population), the total number of V_H genes must be considerably larger.

Several partial sequences of mouse myeloma V_H regions from myeloma antibodies with V_H markers are known. These examples are included in Figure 1, a genealogical tree generated from the N-terminal 38 residues of 17 BALB/c proteins (Hood *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, in press). The categories of idiotype discussed in the previous section can be related to the genealogical tree of V_H sequences on the basis of the degree of homology between

these proteins. The broadest Idx type known in the mouse, the U10-173 marker (Bosma *et al.*, these abstracts), appears to include several of the V_H branches derived from the sequences of M173, U10, and Y5476. Thus, this markers is associated with a homology region shared by these branches of the V_H tree. It would be predicted that such other V_H regions as S117 that belong on this V_H branch might also have the U10-173 determinant. (The Idx determinant need not necessarily be in the part of V_H used to construct this tree, but could be in a subsequent V_H framework region if the initial framework portion is accompanied by additional framework homologies.)

Other more restricted Idx types found among antidextran or antilevan antibodies encompass fewer branches, as, for example, J558 and 104E or Y5476 and U10. These Idx determinants could reflect regions of homology in either the framework or the combining site, since these Idx determinants are found on examples with related specificities. Finally Idi determinants should be found only on the terminal points of the tree associated with a unique V_H sequence. For example, determinants unique to either J558 or 104E have been found (Hansburg *et al.*, these abstracts), as have determinants between the various antilevan and antiphosphorylcholine antibodies.

Each strain of mouse probably has a characteristic V_H tree, and the degree to which these trees overlap can vary. Strains without the U10-173 marker (Table 1) may lack most or all of the V_H genes represented in this V_H branch and may have their own Idx branch distinct from U10-173. Strains may have related V_H trees but differ only at certain terminal branches representing restricted Idx markers, as, for example, C57BL/6 versus BALB/c, with respect to InuIdx. In this case both strains appear to have the U10-173 branch but differ within this family with respect to the V_H regions, having the homology portion corresponding to InuIdx. Lastly, strains may have the same V_H trees except for certain terminal points represented by Idi differences. For example, C58 and C57L have identical idiotypes in all cases tested except for the $A5A^{Cr}$ marker. It is also conceivable that strains differing at major branches of the V_H tree could share Idi determinants. This situation could come about if different strains evolved similar combining sites in unrelated V_H branches. In this instance, V_H framework differences would be found between antibodies with equivalent combining sites.

The evolutionary pathway leading to the V_H locus may have proceeded according to the models proposed for the C_κ alleles in rat or rabbit (Gutman *et al.* 1975). The simplest form is that each mouse strain inherited essentially the same set of V_H genes, which in turn diverged by limited numbers of mutation. Unfortunately, there is insufficient structural information to determine the plausibility of this idea. V_H structures are generally known only in BALB/c; however, recent sequence studies described in these abstracts by the Capra group have provided information on the V_H structures controlled by V_H-ARS and V_H-A5A from A/J. The partial sequences of these show close homology to certain BALB/c V_H genes, for example, between V_H-ARS and V_H-Dex and between V_H-A5A and V_H-S117. One might imagine that V_H-ARS and V_H-Dex are simple alleles that diverged mainly in V_H hypervariable regions. V_H-A5A and V_H-S117 may, on the other hand, be alleles that diverged in framework regions but retained

the same hypervariable region sequence necessary to produce anti-N-acetyl-glucosamine specificity. This view is not consistent with the genetic studies (see below) that suggest that in spite of the homology, these V_H regions are coded for by pseudoalleles. Therefore, the alternative models considered by Gutman and coworkers (1975) may account for the possibility that the V_H regions of different strains may be totally unrelated, both in the types of V genes and the order of related V genes. Strains may have inherited V_H genes that arose by unequal crossingover, or by deletion and duplication; or strains may have equivalent V_H genes governed by allotype-linked regulatory genes.

According to this classification of mouse idiotypes, rabbit V_H markers (the a allotypes) would fall in the extreme Idx category. The a allotypes are found on from 70 to 90 percent of the V_H population and are associated with a variety of different subgroups. The closest analogy to a allotypes in the mouse is the U10-173 marker, which, however, occurs on only a small percentage (one to three percent) of the V_H population. Recent studies on the sequence of V_H pools from rabbits homozygous for different a allotypes (Cannon *et al.*, these abstracts) have shown that a1 and a3 V_H regions differ by from five to seven framework residues and a2 differs from either a1 or a3 by from 12 to 15 framework residues. These differences are located, at least in part, on the surface of the antibody, as can be inferred from the studies of Ansari and coworkers (these abstracts). In addition, alternative residues can be found at certain of these allotype-determining sites that may be related to the subspecificities of a allotypes (Cannon *et al.*, Mage *et al.*, these abstracts). In terms of the genealogical relationships between V_H regions described above for the mouse, the trees for each a allotype may not overlap to the same extent as seems to be the case for the mouse, thus accounting for these extreme Idx type markers. Overlapping regions could occur for V_H regions lacking a determinants (the a^- population), or single branches may occur between a allotypes that are similar or identical. Such examples would constitute a small percentage of the total V_H or might be expressed only with appropriate immunization. It is suggested (Mage, these abstracts) that such overlapping V_H genes might explain the cases of unusual or latent allotypes (Strosberg 1974, Kindt, these abstracts).

Organization of the Mouse Heavy-Chain Locus

The mouse heavy-chain locus consists of two linked clusters of genes, C_H and V_H. These are linked to the locus controlling the electrophoretic mobility of prealbumin (Taylor *et al.* 1975), but have not yet been assigned to a chromosome. The C_H locus consists of a series of genes controlling different heavy-chain classes and subclasses, and a number of alleles have been identified for each of these genes. Several combinations of C_H alleles (allogroups) have been remarkably preserved in the inbred strains. In crosses between strains with different allogroups, no intra-C_H allogroup recombinants were found in over 3000 backcross segregants (Mage *et al.* 1973). The V_H cluster also consists of combinations of alleles and, as can be seen in Table 2, there are strong associations between these V_H and the C_H allogroups. The combination of alleles within V_H allogroups

Table 3. Recombination Between V_H and C_H Markers

	V_H	C_H	Recombinants Found	Frequency in Percentage and 95 percent Confidence Interval
Rabbit[a]	a	d	2/460	0.43 (0.05–1.58)
	a	e	1/366	0.27 (0.01–1.53)
	a	de	3/1011	0.30 (0.06–0.87)
Mouse	Dex[b]	Ig-1a	8/1949	0.41 (0.20–0.84)
	Dex[b]	Ig-1b	5/843	0.59 (0.19–1.36)
	Inuldx[c]	Ig-1a	2/82	2.44 (0.30–8.53)
	A5A^{+}[d]	Ig-1e	2/82	2.44 (0.30–8.53)
	S117^{+}[d]	Ig-1a	3/78	3.84 (0.80–10.83)
	A5A^{Cr}[d]	Ig-1c	1/72[f]	1.38 (0.04–7.50)
	S117^{Cr}[d]	Ig-1c	3/72[f]	4.16 (0.87–11.70)
	ESE[e]	Ig-1b	8/148[f]	5.40 (2.44–10.85)

[a] As summarized in Table 3 of Hamers-Casterman and Hamers (1975)
[b] Riblet and Weigert, these abstracts
[c] Lieberman *et al.*, these abstracts
[d] Berek *et al.*, 1976
[e] McCarthy *et al.*, these abstracts
[f] These values are derived from studies in recombinant-inbred lines. Each line is the equivalent of four backcross segregants (Taylor *et al.* 1975).

is not as well-preserved as for the C_H allogroups and informative matings have separated V_H alleles by recombination. The general model from these observations is that the V_H allogroups are considerably larger than C_H allogroups. The following sorts of data support this view and suggest a tentative order of certain V_H genes.

Table 3 lists the recombination frequencies found between C_H and various V_H markers in rabbit and mouse. In the rabbit, the frequency between a allotype (V_H) and either d or e allotypes (C_H) suggests close linkage. Since the a allogroups are preserved in the V-C recombinants, the crossover events appear to have occurred between V_H and C_H allogroups in each instance. This finding suggests that in the rabbit the a allogroup and C_H are separated by a region that could code for V or C genes for which alleles are not known, such as the a^- class, or that V and C regions are separated by regions not coding for immunoglobulin. In the mouse, using V_H markers for either individual V genes (Idi) or related clusters (Idx), different recombination frequencies between V_H and C_H have been found. These range from about 0.50 for V_H-Dex and C_H (Riblet, these abstracts) to about 5.4 between V_H-ESE and C_H (McCarthy, these abstracts), with intermediate values for other V_H markers (Table 3). These various frequencies imply that the distances of individual V_H genes from C_H are different.

Most analyses of V_H genes have concentrated on two factor crosses to determine the degree of linkage of V_H to C_H. A number of recombinants that arose from such crosses have also been tested for the location of the crossover with respect to other V_H genes, as, for example, BB7 and BAB/14 (reviewed by Eichmann 1975 and Riblet *et al.* 1975). The arrangement of V_H genes in these

recombinants, including certain new V_H genes described in these abstracts, is shown in Table 4. Also V-C recombinants identified among the recombinant-inbred lines originating from $(C57BL/6 \times DBA/2)F_2$ mice (BXD lines) have been tested, using several V_H markers (McCarthy, Mäkelä, Berek, Eichmann, and Taylor, these abstracts). Another source of recombinants is standard inbred strains that may have recombined in this region during the course of their construction. Possible examples (Table 4) are AL/N, C57L, and C58, which have V_H allogroups that do not follow the arrangement typically associated with a particular C_H allogroup. For example, as suggested by Taylor (Jackson Laboratories), AL/N may have been derived from a cross between AKR and A. The V_H arrangement of AL/N may be the result of a double crossover in V_H; it suggests association of the ARS and InuIdx markers. C58 and C57L, Ig-1a$^+$, are inconsistent for Pre-C_H as well as V_H-ESE (D in C58) and A5A (A5A^{Cr} in C57L).

The general finding for V_H-C_H recombinants in the mouse is that the crossover event occurred intra-V_H. As a result of such crossingover, individual V_H markers within allogroups can be ordered. Based on the combined data from BAB/14, BB7, AL/N, C58, and C57L, the following order is likely: C_H-Nase(1)-[T15,Dex,S117$^+$]-[InuIdx,ARS]-A5A^{Cr}-[A5A,ABA-HOP,ESE]; and based on the $B \times D$ lines: C_H-A5A^{Cr}-S117^{Cr}-NP-ESE.

Many problems now exist concerning how to determine the gene order and distances between markers. Since it now appears that this will be a very large, complex locus, it is likely that more than one type of gene may be found within it. While most would agree that the predominant gene type will be a structural gene for the V_H polypeptide, there is a view that regulatory genes may also be found, even though their nature is not clearly perceived. Regulators could control the activation of V_H structural genes, V_L gene selection, or the preferential order for V_H gene activation. A given phenotype could then be controlled by two types of gene, structural and regulatory. Regulatory and structural genes that control a common phenotype could map in different positions.

An important operational aspect of the markers linked to IgC_H is that most of them are in fact demonstrated with induced antibody, thus immune responses and the immunological history (original antigenic sins) of the specific individual mouse or mouse strain may play a role, and temper the type of antibody produced and the appearance of the marker. For each response to an antigen, there are a group of clones that may be stimulated. Preferential clones (*e.g.*, ARS, A5A), however, are often evoked, and their regular appearance and response in some strains is the basis for many of the markers. In strains in which the marker clones are not evoked by antigenic stimulation, alternatives may be found and, in some cases, no response or response to another immunodominant group on the antigen characterizes the nonresponder strain.

To complicate matters further, many V_H phenotypes, *e.g.*, PC, Inu, Dex, are associated with more than one V_H chain. In the usual situation, the V_H chains are closely related with microheterogeneous differences, *e.g.*, T15, 603, 167, 511 anti-PC V_H chains or the Inu E109, A47, U61 group, or J558 and MOPC 104E. It is assumed these are controlled by closely linked genes derived

Table 4. Recombinants in the Heavy-Chain Region[a]

Parental Strain	Recombinant	Pre	Ig-1	Nase-1	Nase-2	T15	DEX	U10-173	ARS	A5A^{Cr}	S117^{+}	S117^{Cr}	NP	A5A^{+}	ESE
A/J		o	e	−	+	−	−	+	+		−			+	ND
BALB/c		a	a	+	+	+	+	+	−	−	+		−	−	ND
	BB7	a	a			+	+		−		+			+	
C57BL/Ka		o	b	−	−	−	−				−		+		
BALB/c		a	a	+	+	+	+	+	−		+		−	−	ND
	BAB/14	o	b	−	+	+	+	+	−		+		−	−	ND
C57BL/6		o	b			−	−	+	−	−	−	−	+	−	D
DBA/2		a	c							+		+	−		N
	BXD25	a	b							+		+	+		D
	BXD27	a	c							+		−	+		D
	BXD30	a	c							+		+	+/−		D
	BXD20	a	c							−		−	+		NT
	BXD21	o	b							−		−	−		D
(BAB/14 × C.AL-9[b])F_1 × CAL-9	CB(F5)	a	b				−								
(BALB/c × A/He)F_1 × A/He	ACA356	o	e				+								
	ACA556	o	e				+								
	ACA563	o	e				+								
	ACA631	a	a				−								
	ACA366	a	e				+								
	ACA704	a	e				+								
AKR		a	d			−		−	−	−	−	−	+/−	−	D
	AL/N	o	d		−	−	−	+	+	−	−	−		−	NT
	C57L	o	a			+	+	+		+	+		−	−	
	C58	o	a			+	+	+		−	+		−	−	D

[a] References are given in the text and Table 1

[b] C.AL-9 is an allotype congenic strain constructed by M. Potter. The V_H and C_H allogroup of C.AL-9 is equivalent to AL/N. C.AL-9 appears to have recombined in the Pre-Ig region (Taylor *et al.* 1975) and is Pre[a]

by duplication and divergence of a common ancestor, but this may not be the only explanation.

Because phenotypes depend upon an immune response, other factors could influence the character of the response or nonresponsiveness. These considerations obviate in some cases a simplistic and ideal situation in which two strains immunized with the same antigen respond monoclonally by making Ab to the same immunodominant hapten. There antibodies are composed of homologous V_H and V_L parts but are controlled by different alleles. In this ideal situation, two strains have homologous and allelomorphic V_H structural genes; the product of one comprises an idiotype and the other does not. Many of the markers, however, cannot be interpreted in this way based on currently available data. Two such examples are the T15 (phosphorylcholine) and Dex systems.

Immunization with R36A Pneumococci evokes several species of phosphorylcholine-binding antibody. Sequence analysis of the chains of phosphorylcholine-binding myeloma proteins reveals a cluster of closely related V_H structures and at least three very different V_κ are involved: V_κ603, V_κH8, and V_κ167–511. Claflin and Rudikoff (these abstracts) have shown that antibodies to phosphorylcholine in A/J mice have the same L and H sequences through the first complementarity region, as seen in the BALB/c tumors (603, 167, T15). Antibody obtained from different strains differs in relative proportion of these V_L types by IEF. In BALB/c almost all of the antibody is of the T15-H8 type, while in other strains three patterns—V_κH8, M603, and M167—are found in relatively equal proportions. In a clonotype analysis of the phosphorylcholine response, Gearhart, Sigal, and Klinman (these abstracts) found considerable heterogeneity in most strains, while 77 percent of the clones in BALB/c were T15-H8 in type. The *T15-H8* clone has nonetheless been found in all strains so far. Recent studies have shown that the T15 molecules in BALB/c apparently are not unique to this strain. In the earlier report of Lieberman and coworkers (1975), the T15 marker which appeared in normal serum was probably a quantitative, rather than a qualitative, marker. T15 Id is present in high and low strains; the expression of the T15 Id apparently is regulated in certain strains; thus, what is being measured may be a regulatory, rather than a structural gene.

Immunization with B1355S polysaccharide evokes in BALB/c and several other strains α1,3 dextran-binding antibody composed of V_H chains and λ0 L chains. The response can be greatly amplified by immunization with a strain of *E. coli* B (Hansburg, Briles, and Davie, these abstracts). The preliminary findings with two different antigenic carriers (*E. coli,* B1355S) suggest that some strains, *e.g.,* C57BL, cannot produce anti-α1,3 dextran-type antibodies containing λ0 chains. Loh, Weigert, Riblet, and Hood (these abstracts) demonstrated the presence of λ0 in NZB, another negative strain. It is possible that these strains respond preferentially to other groups on these antigens. Until data from the intense response to *E. coli* in C57BL is available, we must be satisfied with the notion that C57BL lacks a true homologue of the BALB/c V_H (M104E, J558). Further, the Dex marker in BALB/c and other positive strains cannot be identified as a V_H structural gene or regulatory gene as yet. These two examples indicate that much remains to be learned about the nature of the genes

governing some of the phenotypes associated with the V_H region in the mouse, and that it is too soon to establish precise gene orders or maps.

I. V_H Genetics

Idiotypic Determinants on Mouse Anti-Nuclease Antibodies. David Pisetsky, C. Garrison Fathman, and David H. Sachs. Immunology Branch, National Cancer Institute, Bethesda, Maryland 20014.

Rat antisera capable of detecting idiotypic determinants on mouse antibodies directed against staphylococcal nuclease have been prepared. These antisera, produced by hyperimmunizing rats with affinity column-purified antibodies, have been assayed by measuring the inhibition of antibody-mediated inactivation of the enzymatic activity of nuclease. This inhibitory activity has been shown to be an exclusive property of the anti-idiotypic antibodies in these sera, and was quantitatively retained when such sera were absorbed extensively with normal immunoglobulins to remove all reactivity against nonidiotypic determinants. Antibodies to other determinants did not inhibit inactivation. The idiotypic determinants of antinuclease antibodies from strain A/J mice have been shown to be determined by genes linked to the heavy-chain allotype locus by segregation of the allotype and idiotype in a backcross of (B10.A × A/J) × B10.A. In a study of the strain distribution of these idiotypic markers, it was shown that A/J and SJL strains possess unique unrelated idiotypic specificities on their antinuclease antibodies. The A/J and SJL determinants, however, are both present on antibodies prepared in the BALB/c strain. Preliminary studies indicate that the antibodies produced by the CB.20 strain lack both the A/J and SJL idiotypic determinants, while those of the BAB.14 strain possess the A/J idiotype. These results suggest that the genes determining A/J and SJL idiotypic determinants in BALB/c can be arranged in a linear order of variable-region genes, with the genes which determine SJL idiotypic determinants placed closer to the C_H allotype genes.

V_H-ESE: A New Variable-Region Marker. Margaret M. McCarthy, R.W. Dutton, and B.A. Taylor. University of California at San Diego, and The Jackson Laboratory, Bar Harbor, Maine.

The response of mouse spleen cells to two types of sheep erythrocyte (H, high and L, low) is under multigene control. Discriminator (D) mouse strains make a much higher response to extra antigens (E) found on H SRBC than to shared antigens found on both types of erythrocyte. Nondiscriminator (ND) mouse strains respond equally only to the shared antigens. They make a response to these common antigens that is as great as the discriminator response to the extra antigens. Initial studies used the CXB recombinant inbred strain (derived from the BALB/cBy × C57BL/6By cross) as well as the C.B20 and BAB/14 congenic strains [produced by backcrossing a (BALB/cJ × C57BL/Ka)F_1 onto BALB/c. See *J. Immunol. 115*:1327, 1975.] It was shown that the response to the E antigens on H SRBC is controlled by a V_H-region gene which is allotype-linked. CXB D,E,H,I,K and C.B20 mice are all of the discriminator (C57BL/6) type. CXB G,J and BAB/14 are of the nondiscriminator (BALB/c) type. It is assumed that a crossover, separating most of the V_H region from the C_H region, occurred in the BAB/14 mouse. Our data would indicate that the ESE (extra sheep erythrocyte) response gene is included in the vicinity of the genes V_H-DEX, V_H-A5A, and V_H-NP. Further studies with the Taylor BXD RI strains (C57BL/6J × DBA/2J) corroborated this linkage. The BXD studies show that BXD 1,2,5,8,9,13,14,19,21,22,23,25,27,29, and 30 are all of the BL/6 D parental type. BXD 6,11,12,15,16, and 18 are of the DBA/2 ND parental type. Apparent crossovers appear to be between the V_H and C_H regions in BXD 27 and 30. These offer the possibility of mapping V_H-ESE with respect to the other V_H genes. Preliminary analysis indicates that V_H-ESE may be located distal to V_H-DEX, V_H-A5A, and V_H-NP. The responses of BXH mice (the Taylor RI strains derived from a cross between C57BL/6J and C3H/HeJ) have also been studied and additional data are needed. Preliminary experiments show that BXH 4,6, and 7 are of the C3H ND type while BXH 5,8,9,10,11,14,18, and 19 are of the BL/6 D type. The status of BXH 3 is uncertain. At this point it appears that crossovers occurred in BXH 9,18, and 19 and possibly in 3 between the V_H and C_H (*Ig-1*) regions. Further tests will be made using these strains. (Supported in part by Grant ACS IM-1H from the American Cancer Society, United States Public Health, and contract #1 CP 33255 within the Virus Cancer Program of the National Cancer Institute.)

Genetics of β2→1 Fructosan (Inulin) Antibodies in Mice. R. Lieberman, M. Potter, and W. Humphrey, Jr. Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, and Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

Antibodies to *Aerobacter levanicum* levan in BALB/c mice shared idiotypic specificites with β2→1 fructosan (inulin)-binding myeloma proteins of BALB/c origin while comparable antibodies elicited in C57BL did not. While normal serum from most mouse strains exhibited appreciable titers of bacterial antilevan some strains showed low, while other strains howed high titers of anti-inulin antibodies. Immunization with *Aerobacter levanicum* induced higher titers of antibodies to levan but not to inulin. The finding of two strains—one whose antibodies carried the inulin idiotype (InuIdx) and one that did not—provided a system in which the genetics of the idiotypic marker (InuIdx) could be studied. An F_1 cross of BALB/c and C57BL showed the presence of the InuIdx markers in all the progeny, indicating the dominance of the marker. The availability of the recombinant inbred (RI) and Ig congenic strains related to both BALB/c and C57BL permitted linkage studies of the InuIdx markers to the IgCH allotype locus. Markers were found in the RI strains C×BG and C×BJ and also Ig-congenic BAB 14 and BC-8, but not in RI strains C×BD, C×BE, C×BH, C×BI, and C×BK, nor in Ig-congenic CB20. These findings suggest that the BALB/c phenotype InuIdx is linked with the BALB/c IgCH allotypic markers. BAB 14 has a^2 allotype and VH of BALB/c.

A strain survey for InuIdx markers showed an unusual distribution by being present in strains of three different allotype groups: a^1 (C57L, C58, C3H, CBA, and PL), a^3 (RIII), and a^4 (A/He and AL); and being absent in C57BL/6, C57BL/10, DBA/2, AKR, and NH. Progeny tests were carried out to study the genetics of the InuIdx marker. 168 C57BL×(C57BL×BALB/c)F_1 mice were typed for the InuIdx marker and for allotype. Two of 82 mice of the a^2a^2 allotype carried InuIdx. Both mice were mated to DBA/2, which are negative for InuIdx markers and have the a^3a^3 allotype. All progeny were a^2/a^3, indicating correct parental typing, and six of 15 carried the InuIdx marker. We concluded from these findings that the parental a^2a^2 strains (11265 and 11265 and 11364) are recombinants with C57BL allotype (IgCH) and BALB/c InuIdx loci. Of the 86/168 a^1a^2 backcross mice, 68, as expected, were positive for InuIdx, while 18 were not. Twelve of 18 mice had normal a^1 allotype levels [HI titer 12–13 (log)]. Three of these have been progeny tested by mating to DBA/2. Nine of the 12 a^1a^2 mice obtained had the InuIdx marker, while three did not. From these findings we conclude that the three parents were InuIdx-positive. The reason for the lack of expression of the InuIdx in the other three mice is unexplained. Six other a^1a^2 backcross progeny were also unusual inasmuch as they exhibited low levels of the a^1 allotype [HI titer (log 2) 2–4] in addition to the absence of the InuIdx. None of these has been progeny tested. Presently the data indicate a minimal crossover frequency in two of 168 mice or 1.3 percent.

Disturbances in IgG Regulation in BALB/c.Ig^b Mice (BAB/14 and BAB/20). Andrew Dowsett, Paul Teem, Leonore A. Herzenberg, and Leonard A. Herzenberg. Department of Genetics, Stanford University School of Medicine, Stanford, California 94305.

Confirming and extending the observation of Bosma and Bosma that many BALB/c.Ig^b mice of late backcross generations have low levels of Ig-1b, we have found that BAB mice are subject to several regulatory phenomena which are not observed in either C57BL or BALB/c mice.

We have found a significant decrease in the levels of serum IgG in BAB/14 females during pregnancy and lactation. The levels of γG_1 decreases fivefold and the levels of γG_{2a} decrease twentyfold from normal. The lowest levels of maternal IgG occur approximately one week after the birth of the litter. Experiments in progress indicate that there is a parallel decrease in the numbers of splenic IgG plasma cells. No corresponding decrease in the serum levels of IgM during pregnancy has been observed. Furthermore, no decrease of serum IgG has been observed in BAB/14 mice made pseudopregnant.

By four weeks of age, mice of most strains are producing detectable levels of their own IgG. However, the onset of IgG synthesis in BAB/14 mice does not occur until they are five to nine weeks of age. Since BAB/14 mice receive almost no IgG from their IgG-depressed mothers while nursing, they spend the first five to nine weeks of their lives with extremely low levels of IgG.

Finally, (BAB/14×BALB/c)F_1 mice show a wide variability in serum levels of Ig-1b. No

such variability has been observed for Ig-1a. Of 67 such F_1 mice five to nine months old, five had no detectable (<0.1% normal) serum Ig-1b. Serum levels of Ig of the other allotype and classes were within normal limits. The five mice had normal numbers of spleen cells with membrane Ig-1b. Thus we are attempting to determine if the low levels of serum Ig-1b result from spontaneous T-cell suppression similar to that inducible in (SJL × BALB/c)F_1 mice by perinatal exposure to anti-Ig-1b allotype.

S 117, A Second Idiotype with Specificity to Group A Streptococcal Carbohydrate in Mice. C. Berek and K. Eichmann. Institute for Genetics, Köln, Federal Republic of Germany.

A study of the genetics of the idiotype of the BALB/c myeloma protein S 117, which has specificity for N-acetyl-glucosamine, the major antigenic determinant of A-CHO, was carried out. Only a small portion of the antibodies elicited in BALB/c mice by immunization with Group A streptococci carry idiotypic determinants in common with the BALB/c myeloma protein S 117. The expression of these idiotypic determinants is under the control of a gene which is linked to the *Ig-1*$^{a+}$ allotype locus. Gene S 117$^+$ and the gene A5A$^+$, which controls the expression of the A5A idiotype of the antibody response to A-CHO in strain A, were shown to be codominantly expressed in (A × BALB/c)F_1 mice. Using the recombinant strain BB7, we could show that genes S 117$^+$ and A5A$^+$ map at nonallelic positions in the Ig-1 complex. The expression of antibodies crossreactive with idiotype S 117 and idiotype A5A was found to be linked to the *Ig-1*^{c} allotype. Using recombinant inbred strains BXD, we could show that these crossreacting antibodies are under the control of two different genes (S 117^{Cr}, A5A^{Cr}), which can be separated by recombinational events.

Recombination data suggest multiple loci for V_H genes encoding specificity for A-CHO. This indicates complex pseudoallelic relationships between the genes coding for the expression of anti-A-CHO antibodies in different *Ig-1* haplotypes.

Fine-Specificity and Isoelectric Focusing Characteristics of Anti-ABA-HOP, Anti-NBrP, Anti-NClP, and Anti-NP Antibodies. O. Mäkelä, Marja Julin, and Klaus Karjalainen. Department of Serology and Bacteriology, Helsinki University, Helsinki 29, Finland.

Fine-specificity of anti-ABA-HOP (azobenzenearsonate coupled to carbon atom 3 of 4-hydroxy-phenylacetic acid) was studied by determining the relative affinity to two related compounds, ABA-MIP (additional I in carbon atom of 5 HOP) and ABS-HOP (sulfonic acid in the place of the arsonic acid of ABA-HOP).

Individual mice were placed in a plane defined by the coordinates (the two relative affinities). Mice of a particular strain occupied a characteristic area in the plane. One area was occupied by A/J mice; another by CBA, C3H, and AKR mice; the third area by C57BL/6 mice; and the fourth (overlapping with the third) by BALB/c mice. C57BL/6 antibody, besides having a characteristic fine-specificity, also had a characteristic IEF pattern that was shared by many individuals.

Inheritance of the four (idio)types was studied in families. A total of 258 backcross mice were tested. Five of the six tested backcrosses indicated that an allotype-linked gene exclusively controlled the idiotype but the sixth, (C57BL/6 × CBA) × C57BL/6, suggested that an allotype-unlinked gene also played a role.

Two superidiotypes were described in the mouse. Both are characterized by an IEF pattern and a fine-specificity. One of them (N-7) is produced by most or all mice of strains BALB/c, RF, RIII, and IAH upon immunization with either NBrP, NClP (*J. Exp. Med. 141*:840, 1975). The other (N-6) was produced by all CBA and C3H mice upon immunization with NBrP or NClP.

Both N-3 and N-4 seem to be under the control of a V_H gene.

Presence of T15-S63 Idiotype on M511. R. Lieberman and M. Potter. National Institutes of Health, Bethesda, Maryland 20014.

Sakato and Eisen (*J. Exp. Med. 141*:1411, 1975), using radioimmunoassay methods, reported crossreactions between T15 and M511. In an earlier study (Potter and Lieberman, *J. Exp. Med. 132*:737, 1970), we failed to find precipitation of M511 by anti-T15 antisera (Table 1). With the more sensitive HA method, we can show that T15 and M511 share an idiotype (IdX determinant). SRBC coated with T15, H8, M511, M167, and Mc603 were reacted with idiotypic antiantisera

Table 1. Detection of U10-173IdX in Mouse Strains of Different Allotypes[a]

Allelic Group	U10-173IdX$^-$	U10-173IdX$^+$
a^1 (a)	C3H, PL/J, CBA	BALB/cJ (200 μg/ml) C57L/J, P/J, BALB/c, 129/J (66–125 μg/ml) C58/J, C57BR/cdJ, SEC/ReJ (60–65 μg/ml) BUB/BnJ, SEA/J, ST/bJ (25–30) μg/ml)
a^2 (b)		SJL/J (94 μg/ml), C57BL/6 (31 μg/ml) C.B-17 (58 μg/ml), LP/J (12 μg/ml)
a^3 (c)		RF/J (100 μg/ml), SWR/J (125 μg/ml)
a^4 (d) (d)	AKR	AL/N (79 μg/ml) NZB (10 μg/ml), A/J (57 μg/ml)
a^5 (f)		CE/J (65 μg/ml)

[a] The sensitivity of the assay was such that we could detect five μg/ml of U10-173IdX. Allelic groups are designated according to Lieberman and Dray (*J. Immunol. 93*:584, 1964) and in parentheses according to Herzenberg *et al.* (*Ann. Rev. Genet. 2*:209, 1968).

to the respective proteins. The anti T15 and H8 idiotypic antisera strongly agglutinated M511-coated SRBC, while anti-M511 idiotypic antisera only weakly agglutinated T15-coated SRBC but gave very strong agglutination to itself. Many idiotypic antisera in our experience show strong agglutinating activity but fail to precipitate.

These findings indicate that T15-S63 idiotypes were found on phosphorylcholine-binding myeloma protein T15 and on M511, which have different L-chain subunits. The location of the T15-S63-M511 IdX is not fully determined. Since the L chains in these proteins are so different, it is probably a V_H determinant. In fact, the sequences of M511 V_H and T15 and H8 V_H have only two or three differences.

These findings raise the following questions. Do the idiotypic antibodies raised to T15 or H8 identify different specificities? Is it a nonsite determinant on S63-T15 which is genetic, *i.e.*, in BALB/c but not in C57BL/6? Is it a site-related determinant which is probably not genetic. It is not known whether the T15-M511 determinant is site-related.

These findings indicate that T15-S63 idiotype can be found in M511, a phosphorylcholine-binding myeloma protein with different L-chain subunits. Thus the T15-S63 idiotype, which is not associated with the binding site, is in fact a cross crossspecific idiotype controlled by the V_H.

A New Heavy-Chain Marker in Mice. M.J. Bosma, C. De Witt, S.J. Hausman, R. Marks, M. Potter, and B. Taylor. The Institute for Cancer Research, Philadelphia, Pennsylvania, National Cancer Institute, Bethesda, Maryland, and The Jackson Laboratory, Bar Harbor, Maine.

Repeated injections of the IgG_{2a} F_{ab} fragment of a mouse myeloma (MOPC 173) into a rabbit resulted in an antiserum that, after absorption with several unrelated myeloma proteins, was specific for the 173 idiotype (173Id). About 20 percent of the anti-173Id antibodies crossreact with the IgG_{2a} produced by another myeloma, UPC 10 (U10). Using a radioimmune assay and separated heavy (H) and light (L) chains of 173 and U10, we were able to show that the crossreactive specificity of U10 (U10-173IdX) is on the H chains alone.

The screening of over 100 mouse myeloma proteins showed that U10-173IdX is present on a small number of immunoglobulins (Igs) having different antigen combining specificities. These U10-173IdX$^+$ proteins, including 173 and U10, can be grouped according to their antigen-binding specificities (in parentheses): U10, ABE 48, Y5476 (2–6 levan); J539, X24, X44, T601, CBPC 4 (β-1-6-D-galactan, other members of this group have yet to be tested); MOPC 406 (N-acetyl-D-mannosamine); MOEV 48 (specificity unknown); and 173, PC 3 (these two proteins give the same isoelectric focusing patterns but their antigen-binding specificities are unknown). All of the above are members of the IgA class except 173, U10, and PC 3 which belong to the IgG_{2a} subclass.

As shown in Table 1, a survey of 25 different inbred mouse strains revealed that U10-173IdX is detectable in most strains in the range of 30–100 μg/ml of serum. This represents about one percent of the total serum Ig concentration. Four inbred strains have no detectable U10-173IdX; these are C3H, CBA, PL/J, and AKR. From the segregation of U10-173IdX in recombinant inbred lines derived from F_2 crosses of (AKR × C57L), it is clear that the inheritance of U10-173IdX is linked to the H-chain allotype.

Immunization of C3H·SW-*Ig*^{b} mice with 50 μg of levan from *Aerobacter levanicum* resulted in a specific increase of the U10-173IdX serum concentration from 30 μg/ml to 700 μg/ml. Absorption of these immune sera with levan-conjugated Sepharose beads (a gift from N. Glaudeman) lowered the U10-173IdX concentration to ~175 μg/ml. U10-173IdX was not detectable in C3H·SW-Ig^a mice before or after immunization with levan, even though C3H·SW-Ig^a mice all make precipitating antibody to levan.

We are led to conclude that U10-173IdX is the equivalent of a V_H allotype that serves as a marker for one or more V_H subgroups. The rationale for this statement is as follows: the distribution of U10-173IdX among different inbred strains does not correlate with any known mouse allotypes; U10-173IdX is found on a few IgA and some IgG_{2a} myeloma proteins; U10-173IdX is apparently associated with different families of myeloma proteins having different antigen-combining specificities; and when one of these antigens (levan) is used as an immunogen in mice of the appropriate C_H allotype, the U10-173IdX concentration is specifically elevated twentyfold. Families of myeloma proteins representative of different antigen combining specificites are presumably encoded by different V_H germline genes. According to this reasoning, U10-173IdX must correspond to at least three, and possibly five, different V_H genes. Since U10-173IdX represents about one percent of the total serum Ig in normal mice, there could be as many as 300 to 500 V_H genes in the germline. Alternatively, U10-173IdX may correspond to one (of a ~100 V_H genes) germline gene which, as a result of somatic diversification, is responsible for many different antigen-combining sites. (Supported by USPHS grants CA-04946, CA-06927, and RR-05539 from the National Institutes of Health, contract #1 CP33255 within the Virus Cancer Program of the National Cancer Institute, and by an appropriation from the Commonwealth of Pennsylvania).

II. V_L Markers in the Mouse

Attempts to Obtain Mouse Ig Fractions Enriched in the I_B-Peptide Marker, a V_L-Region Polymorphism. Paul D. Gottlieb. MIT Center for Cancer Research and Department of Biology.

We have attempted to obtain populations of IgG and L chains which are enriched in the I_B-peptide marker, a genetic marker found in mouse V_L regions (Edelman, G.M. and Gottlieb, P.D.: *Proc. Natl. Acad. Sci. U.S.A. 67*:1192, 1970). Genetic linkage of this marker has been demonstrated with the locus on chromosome 6 (linkage group XI) which governs the Ly-3.1 surface antigen on thymic lymphocytes (Gottlieb, P.D.: *J. Exp. Med. 140*:1432, 1974). Preparations enriched in I_B-positive L chains would provide material for structural studies of amino acid sequence diversity in portions of their V_L regions other than the neighborhood of Cys I. They may also provide suitable antigens for preparation of antisera specific for this V-region polymorphism.

Purified serum IgG of the I_B-positive AKR/J strain was subjected to ion exchange chromatography on DEAE-Sephadex. Peptide maps of L chains from several representative fractions were prepared, and the regions corresponding to the I_A-I_B complex of Cys I hexapeptides were further subjected to high voltage paper electrophoresis at pH 10.6. The latter procedure separates peptide groups I_{A1} and I_B, which terminate in lysine from the homogeneous peptide I_{A2}, which terminates in arginine. A slight enrichment in light chains containing the I_B-peptide group was apparent in the early (more positively charged) fractions, while the quantity of I_{A2}-positive L chains in these fractions was considerably diminished. The more retarded fractions contained virtually no I_{A1} and I_B-positive L chains and were substantially enriched in I_{A2}-positive chains.

AKR/J Fab fragments were fractionated by starch zone electrophoresis, and the Fd fragments and L chains of individual fractions were separated by ion exchange chromatography on SP-Sephadex in 8 M urea. All Fab fractions contained equivalent amounts of I_B-positive L chains, indicating no enrichment by such procedures. Ion exchange chromatography of purified AKR/J L chains under similar conditions yielded several fractions which contained equal amounts of I_B-

positive L chains and which chromatographed in the position of κ chains. Investigation of a number of antihapten and anti-BSA antibodies failed to yield significant enrichment in I_B-positive L chains, and L chains from homogeneous antistreptococcal group A carbohydrate antibodies from the AKR/J strain (kindly provided by Dr. K. Eichmann) were devoid of I_B-positive L chains. Finally, AKR/J IgG2a was purified by affinity chromatography of AKR/J IgG on columns of rabbit anti-mouse IgG2a/Sepharose, and peptide maps of L chains from IgG2a and from IgG2a-depleted fractions were prepared. Both fractions contained equivalent amounts of I_B-positive L chains.

Thus, I_B-positive L chains appear to be slightly more prevalent in the more positively charged IgG species, but otherwise behave like a heterogeneous population of mouse κ chains. Selective enrichment of I_B-positive L chains by immunization has not yet been achieved. In anticipation of this eventuality we had already begun to place the I_B-peptide marker on the BALB/c genetic background by typing successive backcross generations for the closely linked Ly-3.1 thymocyte surface antigenic specificity. We are at present inbreeding these mice after seven backcrosses to BALB/c, and we will attempt to obtain homogeneous I_B-positive immunoglobulins by inducing and screening plasmacytomas in this strain. (This work was supported in part by U.S. Public Health Service Research Grant CA 15808 from the National Cancer Institute to Paul D. Gottlieb, and by U.S. Public Health Service Research grant CA 14051 from the National Cancer Institute to the Massachusetts Institute of Technology Center for Cancer Research.)

Genetic Polymorphism of Mouse Immunoglobulin Light Chains Revealed by Isoelectric Focusing. D. Gibson. Department of Biochemistry, University of Sherbrooke, Sherbrooke, Quebec, Canada.

Light chains isolated from normal nonimmune immunoglobulin give rise to a highly detailed and species-specific banding pattern when analyzed by isoelectric focusing in polyacrylamide gels. The species-specific nature of the focusing patterns was studied by analyzing reduced and alkylated light chains from individual animals and birds of a variety of species. Differences could be noted between species as closely related as human and rhesus monkey.

Analysis of the light chain fraction of immunoglobulin isolated from nonimmune serum of nine different inbred mouse strains led to the discovery of a genetic polymorphism involving several of the focusing bands (estimated to represent two to five percent of the total light chain). Strains SWR/J, C3H/HeJ, C57Bl/6J, DBA/1J, CBA/J, and A/J showed virtually identical banding patterns, while strains C58/J, AKR/J, and RF/J shared at least two focusing bands differing from the other six mouse strains. In addition, differences were also noted between strains RF/J and C58/J, indicating that more than one marker may be involved. Analysis of the light-chain focusing patterns from individual SWR/J, C58/J, and F_1 hybrid mice obtained from the C58 × SWR cross indicated that the differences in the banding pattern were simply inherited and were expressed codominantly. The fact that the polymorphism affected only a small proportion of the light-chain population suggested that it is most likely associated with the variable region of the light chain. An alternative explanation which cannot be ruled out at the present time is that it might be caused by polymorphism involving the C region of the lambda chain.

A simple and rapid procedure was developed for the isoelectric focusing analysis of immunoglobulin light chains starting with small samples of serum. The procedure involved preparation of light chains from reduced and alkylated immunoglobulin by electrophoresis in 8 M urea-formate acrylamide gels. In this way, light chains could be prepared in microquantities from up to 10 immunoglobulin samples simultaneously. The light-chain region of the acid-urea gel was then applied directly on the surface of the 6 M urea-polyacrylamide isoelectric focusing gel for analysis by isoelectric focusing. Drying of the applied sample-containing gel strip was prevented by covering the isoelectric focusing gel with a layer of petrolatum.

In collaboration with Dr. B.A. Taylor and Dr. M. Cherry of the Jackson Laboratory, Bar Harbor, the isoelectric focusing phenotypes of the light chain of a number of AKXL recombinant inbred mice were examined.

In the 16 AKXL strains examined so far, a complete concordance has been found between the presence of the AKR-associated Ly-3.1 antigen and the AKR-like light chain focusing pattern. This result, as well as the strain distribution of the unique focusing patterns (present in Ly-3.1 positive strains AKR, C58, and RF), strongly suggests that the light chain marker described here is closely linked to the Ly-2.3 locus of the mouse. (This research was supported by the Medical Research Council of Canada, Grant #MT-4317.)

Genetic Linkage of a Kappa Chain Variable-Region Marker to the Ly-2,3 Alloantigen Complex on Mouse Thymocytes. J.L. Claflin, M. Kopchick, B. Taylor, and M. Cherry. University of Michigan Medical School, Ann Arbor, Michigan, and The Jackson Laboratory, Bar Harbor, Maine.

The immune response to phosphorylcholine (PC) in mice is characterized by a small, well-defined set of antibodies (all IgM,K), one member of which is idiotypically indistinguishable from one of the PC-binding myeloma proteins, HOPC 8 (H8). Light (L) chains of reduced and alkylated H8-like mouse anti-PC antibodies, subjected to analytic isoelectric focusing in a polyacrylamide gel, migrate in the same restricted pH range as do H8 L. However, careful analysis of the isoelectric focusing patterns revealed that in some strains the H8-like L chains focus slightly more anodally than those of others. The L chains of H8-like anti-PC antibodies from C57L and C57BL/6 mice show the same degree of microheterogeneity and migrate to the same positions as do the H8 L chains, pH 7.3–8.2. By contrast, the L chains of anti-PC antibodies from AKR and PL mice focus distinctly more anodally, pH 7.15–8.1. The structural variation causing the differences in isoelectric point, pI, of these H8-like L chains, termed κPC8, produces two variants, AKR type or A and C57L type or B. The most probable location of the κPC8 marker is the variable region, since the kappa chains of other idiotypically related antibodies in C57L and AKR do not show differences in their electrophoretic mobility. A survey of immune sera of inbred strains showed that the κPC8-A marker was present in AKR, RF, PL, and C58, and that κPC8-B was present in C57L, BALB/c, CBA, C3H, MA, ST, 129, SEC, C57BL/6, DBA/2, AL, A, and CE mice. Examination of (AKR × C57L)F_1 and (AKR × DBA/2)F_1 showed that κPC8-A is dominant. This strain distribution pattern of κPC8 suggested an association with the Ly-2,3 alloantigenic surface marker of mouse thymus-dependent lymphocytes. Evidence favoring linkage to *Ly-2,3* came from studies of the recombinant inbred lines AKXL (RI from AKR × C57L), HP mice (RI from AKR and C57BL/6), LT mice (RI from C58 × BALB/c), and the C57BL/6.PL (*Ly-2,3*) congenic strain. (Supported by USPHS NIH grant #A112533).

Structural and Functional Differences between BALB/c and NZB Myeloma Proteins. E. Loh, M. Weigert, R. Riblet, and L. Hood. Division of Biology, California Institute of Technology, Pasadena, California, and Institute for Cancer Research, Philadelphia, Pennsylvania.

The myeloma system of the BALB/c mouse has been most revealing regarding the genetics, structure, and biology of the antibody molecule. However, the BALB/c myelomas appear to be selected by a variety of criteria in comparison to the distribution of normal serum immunoglobulins. Thus it should be useful to look at the diversity of the myeloma proteins of a second mouse strain. This study has investigated myeloma proteins which were raised in the NZB strain with respect to antigen binding, class distribution (see Herbert Morse III *et al.*, these abstracts), and the N-terminal sequences of both light and heavy chains. Comparison of these results with the BALB/c myelomas suggests that the myeloma proteins from the NZB strain come from a different but overlapping pool of immunoglobulin-synthesizing cells.

Two NZB myeloma proteins bind α1,6-dextran and one binds α1,2 levan, antigens which certain BALB/c myeloma proteins also bind. Twelve myeloma proteins have been found to bind DNA, an antigen which none of the BALB/c myelomas binds. The NZB tumor proteins studied do not bind Dnp or phosphorylcholine, two haptens which many BALB/c myeloma proteins bind. NZB tumors are predominantly of the IgG class, in contrast to the class distributions of the BALB/c tumors, which are predominantly of the IgA class. Thus the antigen binding and class distributions of the NZB and BALB/c myeloma proteins appear to be quite distinct.

The sequences for the N-terminal 23 residues of 25 randomly chosen κ chains have been determined. Nineteen different sequences were found, one of which has already appeared among the BALB/c κ sequences. This sequence derived from three different NZB tumors (2880, 3741, 1229), has already been seen in four different BALB/c tumors (M321, T124, BFPC32, M70). In the genealogical tree constructed from the N-terminal κ sequences of BALB/c and NZB myeloma tumors, two clusters of three NZB light chains have been found, which differ from BALB/c sequences by at least three base changes in the first 23 residues (possibly strain-specific subgroups). Hence, the NZB κ sequences are extremely diverse and generally appear to be distinct from those of their BALB/c counterparts. Indeed, the fact that only one sequence has been found that is shared by both the BALB/c and NZB mice suggests that the two sets of light chains come from distinct lymphocyte populations.

The size of the first hypervariable region has been determined for 11 κ chains. Four distinct size classes were found. A correspondence was found between the N-terminal sequence and the size of the first hypervariable region.

Two-thirds of the heavy chains from the NZB tumors are blocked at their N termini. In BALB/c tumors, heavy chains rarely have blocked N termini. The genealogical tree of the heavy-chain N-terminal sequences from the NZB and BALB/c myelomas shows that the proteins from one strain are not randomly distributed with regard to the proteins of the second strain. These differences lend further support to the supposition that the NZB and BALB/c tumors were drawn from different lymphocyte populations.

III. Structural Correlates

Structural Correlates of Rabbit V_H Allotypes. L.E. Cannon, M.N. Margolies, M.S. Rosemblatt, and E. Haber. Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts.

Examination of V_H-region amino acid sequence data from homogeneous rabbit antibodies and from rabbit-pool Ig has revealed three segments of V_H that are most likely to contain the structural correlates of the group *a* allotypes. These segments were located at positions 4–16, positions 63–73, and positions 84 and 85. We report amino acid sequence data for three homogeneous allotype a1 H chains derived from antipneumococcal type III antibodies 3381, 3374, and 3T72. These sequences are compared for the allotype-related segments with previously published sequence data from allotype a1, a2, and a3 H chains. For the 24 positions compared, allotype a1 and a3 H chains are most closely related (five to seven residue differences). Allotype a2 H chains differ from both a1 and a3 H chains by 12 to 15 residues. H chains of a single allotype differ by zero to three residues from one another, and these differences are restricted to positions 14–16 and 85. There are three different sequences at positions 14–16 among a1 H chains (Gly-Thr-Pro, Thr-Pro-Gly, and Gly-Gly-Ser) and, as previously shown by Mole, a single Ala/Gln interchange at position 85 among a2 H chains. Crystallographic models indicate close proximity of positions 14–16 with positions 84 and 85. The demonstration of amino acid sequence differences in these segments among H chains of a single allotype may represent the structural correlates of group *a* subspecificities. While it is not yet possible to assign specific residue loci to allotypic determinants, the a1, a2, and a3 allotypes can be distinguished from one another by sequence, as well as by serological methods. (Supported by a Grant-in-Aid from the American Heart Association and NIH Grant AI-04967).

Immunological and Structural Properties of Peptides with a3 Allotypic Determinants. A.A. Ansari, E. Appella, M. Carta-Sorcini, and R.G. Mage. National Institute of Allergy and Infectious Diseases, and National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

Rabbits express a greater variety of allotypes than any other animal studied thus far. Further, rabbit (and other *Leporidae*) immunoglobulins (Igs) bear allotype markers in the variable portions of their heavy chains. These markers (a1, a2, and a3) offer a unique opportunity to study Ig variable-region (V) genetics through the use of allotypes. Their structural definition is of decisive importance for understanding the genetic basis of heavy-chain variable regions.

We have isolated an immunopeptide bearing a3 allotypic determinant(s) from the γ chain of an a3 homozygous rabbit immunized with type III pneumococcal vaccine. The isolation procedure involved citraconylation of the H chain, tryptic digestion, and passage of the digest through an anti-a3 immunoabsorbent column either directly or after an intermediate step of Sephadex G-75 chromatography. The bound peptides (T1) were eluted with 0.1 M acetic acid, further digested with trypsin, and again passed through the anti-a3 immunoabsorbent column to purify the bound immunopeptide T2.

Quantitative measurements of the immunological activity of the immunopeptide T2 and of the intermediate products in the isolation procedure were done using a radioimmunoassay that involved inhibition by these peptides of a reaction between ^{125}I-labeled anti-a3 antibody and Sepharose-bound a3 IgG. T2 could inhibit the a3-anti-a3 reaction by more than 60 percent. Thus, this immunopeptide had major a3 determinant(s).

The molecular weight of T2 was $\sim$6000 which decreased to $\sim$3000 after reduction and alkyla-

tion. These data, together with NH_2- and COOH-terminal analyses and cysteine peptide mapping, demonstrated that T2 is composed of two polypeptide chains linked by a disulfide bond, one from the cysteine 22 region having lysine at the COOH-terminus, and the other from the cysteine 92 region having arginine at the COOH-terminus. The lysine peptide had ~22 residues and started from position 17 with the sequence: Gly-Asx-Glx-Ser-Thr-Cys. The framework sequence from 17 to 20 is different from those reported so far. In addition, the heavy chain used in these studies had some unusual features, including a histidine in the first hypervariable region. The other peptide was about 30 amino acids in length and ended with arginine 94. It included positions 84–85 that are believed to have substitutions correlating with *a* allotypes.

Residues 17–33 of the lysine peptide and 64–79 and 84–85 of the arginine peptide were found to be fully exposed on the surface in a hypothetical three dimensional model (D.R. Davies and E.A. Padlan, *Proc. Roy. Soc. Med. Symp., Oct. 20–22,* 1975, Rockefeller Univ., Raven Press, New York, in press) of the Fv portion of another rabbit anti-SIII antibody BS-5 (J.-C. Jaton, *Biochem. J. 143*:723, 1974). Moreover, these residues were far removed from the antibody-combining site.

Complex Allotypes and Their Evolutionary Implications. L. Hood. Division of Biology, California Institute of Technology, Pasadena, California 91125.

We have recently suggested that alleles (or allotypes) can be divided into two categories (G. Gutmán, E. Loh, and L. Hood, *Proc. Natl. Acad. Sci.* U.S.A.: *72*:5046, 1975). Alternative forms of *simple allotypes* segregate in a Mendelian fashion in mating studies and differ by one or a few amino acid substitutions (*e.g.,* the *Inv* marker of the human chain). In contrast, alternative forms of *complex allotypes* differ by multiple amino acid residues and generally segregate in a Mendelian fashion. We have recently analyzed two C allotypes that fall into the complex category. The C_κ regions from rabbits of differing allotypes (*i.e.,* b4 and b9) differ by 33 amino acid substitutions and three sequence gaps (V. Farnsworth, R. Goodfliesh, S. Rodkey, and L. Hood, *Proc. Natl. Acad. Sci.* U.S.A., in press). Likewise, partial sequence analysis of the gene products encoded by two rat κ alleles demonstrates that the corresponding C_κ regions differ by ten amino acid substitutions and one sequence gap (G. Gutmán, E. Loh, and L. Hood, *Proc. Natl. Acad. Sci.* U.S.A. *72*:5046, 1975). Moreover, these differences are scattered throughout the corresponding *C* regions. By the definitions given above, both of these immunoglobulin allotypes are complex allotypes.

The importance of the distinction between simple and complex allotypes lies in the very different types of genetic or evolutionary mechanism implied. Simple allotypes are probably coded by alternative alleles at a single structural locus. In contrast, complex allotypes may be explained by one of three genetic models:

1. Complex allotypes may evolve by the divergence of alleles at a single genetic locus. If so, intense selective pressures are required to fix many substitutions in relatively short periods of evolutionary time.

2. Complex allotypes may evolve by gene duplication, mutational divergence, and subsequent crossingover events that contract the gene number to one. In different populations (*e.g.,* inbred strains) different genes could remain.

3. Complex allotypes may evolve by gene duplication with a control mechanism that permits them to be expressed so that they mimic a Mendelian pattern of genetic segregation. The important point is that these immunoglobulin allotypes may have evolved by any one of these three mechanisms, and various experimental approaches will allow one to distinguish among the possibilities.

Structural Studies on Induced Antibodies with Defined Idiotypic Specificities: Determination of the V_H Subgroup of Mouse Anti-Ars and Anti-Streptococcal Antibodies. J.D. Capra, K. Eichmann, C. Berek, A. Tung, and A. Nisonoff. The University of Texas Health Science Center, Dallas, Texas, and Institute of Genetics, University of Köln, Federal Republic of Germany.

The murine V_H genetic map consists of two different subloci of V_H genes. Seven idiotypes have been allocated to these two different subloci, one of which contains genes coding for the A5A idiotype, while the second contains the genes encoding for six other idiotypic markers. Previous studies from this laboratory have shown that anti-Ars antibodies are best classified in the murine V_HII subgroup. Studies from other laboratories indicate that the J558 idiotype could be classified in a similar fashion. The sequence of the T15 myeloma protein V_H region is most characteristic of the murine V_HIII subgroup. The present studies were undertaken in order to study the V_H subgroup

of two additional murine V_H markers, A5A and S117. Since one of these markers (A5A) has been allocated to a separate sublocus, comparisons can be made for the first time between the framework residues (V_H subgroup) and the murine genetic map. The data indicate that within a single presently defined sublocus, proteins exist which contain remarkably different framework residues.

Two Heavy-Chain Variable-Region Sequences and Partial Light-Chain Sequences From Levan-Binding Myeloma Proteins. M. Vrana, S. Rudikoff, and M. Potter. National Cancer Institute, Bethesda, Maryland 20014.

We are presently studying a group of 12 myeloma proteins of BALB/c origin that bind fructosan with the B2→1 linkage (inulin). The heavy-chain sequence of the complementarity regions and 95 percent of the framework from two of the proteins, EPC-109 and ABPC-47N, has been determined. Also, approximately 60 percent of the corresponding two light chains, including L_1 and L_3, have been sequenced.

In the two heavy chains all complementarity regions are identical and four conservative replacements are seen in the framework portion of the molecule. In EPC-109 positions 48, 92, 94, and 97 are Ile, Gly, His, and Thr. In ABPC-47N, these positions are Val, Ala, Tyr, and Ser. The H_3 complementarity region is extremely short, consisting of one amino acid, suggesting that a deletional mechanism has been important in the evolution of the heavy-chain variable regions necessary for levan binding. Three of the four framework changes are just prior to the H_3 complementarity region, again suggesting that the area of DNA coding for this region may be more susceptible to mutagenesis than other areas.

The corresponding two L chains have been sequenced from the N-terminal end to residue 50 and from residue 84 through the end of the variable region. Three amino acid differences have been found. Onc change is located in each of the first and third hypervariable regions and the third difference is in the framework near the end of the variable region. In EPC-109L positions 30, 92, and 106 are asparagine, serine, and lysine, and in ABE-47NL these positions are serine, threonine, and isoleucine.

These sequences are of added interest in that both crossreacting (Idx) and individual (Idi) idiotypes have been identified among this group of proteins. From the three-dimensional model of McPC-603, the interchanges noted in the heavy chain are in positions in the interior of the molecule and presumably could not contribute, by themselves or by interaction with other areas, to idiotypic determinants. The two interchanges in L_1 and L_3 are three-dimensionally very close to one another and are located on the surface of the molecule, making them possible candidates for the idiotypic differences between these two proteins. The interchange at position 106 in the L chain is not near the surface of the molecule. Further analysis of this group of proteins should provide more structural evidence for these idiotypic differences and also reflect the genetic mechanism(s) compatible with the observed structures.

Structure-Function Correlates for DNP and PC Binding Myeloma Proteins. M.W. Hunkapiller, A.M. Goetze, J.H. Richards, and L.E. Hood. Divisions of Chemistry and Biology, California Institute of Technology, Pasadena, California 91125.

Sequence Analysis of DNP-Binding Myeloma Proteins. The heavy chains from three BALB/c myeloma proteins (XRPC-25, J-604, J-688) which bind dinitrophenyl (Dnp) haptens have been isolated and analyzed by automated sequencing techniques. Sequence data for the N-terminal 38 residues of the proteins, when compared to that previously obtained for three other DNP-binding myeloma proteins (MOPC-460, MOPC-315, S-23), reveals two distinct sequence patterns. On the other hand, sequence analysis of the light chains of five of the proteins shows that each is quite distinct from all of the others. The restricted structural diversity of the heavy chains suggests (although the data is as yet limited) that much of the heterogeneity observed in the normal BALB/c response to Dnp may result from light chain diversity.

Nuclear Magnetic Resonance (nmr) Studies of PC Binding to Myeloma Proteins. BALB/c myeloma proteins which bind phosphorylcholine (PC) have at least four heavy-chain and three light-chain sequences, with five different chain combinations represented by TEPC-15, W-3207, MOPC-603, MOPC-511, and MOPC-167. We have analyzed the interactions of these proteins with PC by

carbon and phosphorus magnetic resonance techniques to study the effect of the sequence diversity, since much of it is located in the regions (hypervariable) that contact the hapten. As judged by nmr analysis, binding of the choline methyl groups of PC is strongest for those proteins (MOPC-511 and MOPC-167) which show significant affinity for choline, while TEPC-15 (which binds PC much more tightly than choline) interacts strongly only with the phosphate group of PC. W-3207 and TEPC-15, which have identical heavy chains through residue 100 and different light chains, exhibit significantly different interactions with the choline methyl groups of PC. MOPC-511 and MOPC-167 (similar light and distinct heavy chains) exhibit different interactions with both ends of PC. Thus, both the heavy and light chains can contribute to binding of hapten and to the subspecificity of antibodies with related specificities.

Structural Characteristics of Anti-Streptococcal Group-Specific Antibodies Isolated from Hyperimmune Antisera of Selectively Bred Rabbits. J.G. Braun, H. Huser, and W.F. Riesen. Basel Institute for Immunology, Basel, Switzerland.

Considerable advance has been made with special breeding programs in the selection of rabbits with amplified immune responses to the streptococcal group A-variant polysaccharide. The incidence of restricted and monoclonal high responders (20 to 50 mg of antibody/ml of antiserum) in progeny of specially bred rabbit families is up to 60 to 70 percent. The availability of a large number of these antibodies raised in closely related rabbits has stimulated a search for variable-region markers common to antibodies specific for the same polysaccharide antigen.

Under conditions of analytical isoelectric focusing in polyacrylamide gels (pH 5–10) where specific antibody clones are developed by an overlap with the isotope-labeled A-variant polysaccharide, as many as 50 percent of the monoclonal antibodies may share overlapping isoelectric points when antisera of siblings are compared. These siblings are progeny of the third and fourth generation of selective breeding; their antisera contain between 15 and 25 clonotypes binding the A-variant polysaccharide.

The amino acid sequences of five V_L regions (allotype *b4*) and the partial sequences of 12 additional κ V_L regions from anti-A-variant polysaccharide antibodies were determined. These sequences contain 70 invariant positions, as opposed to only 50 invariant positions in other rabbit V_L regions. Variability within the framework residues lacks randomness, and parent offspring relationship or otherwise close familial relationship is apparent in several instances. Further comparison with other rabbit V_L regions reveals a reduction of variability in the complementarity-determining regions for antigen by 2.3 to 5.5-fold. Residue positions 50–56, known to mark the second hypervariable region in human κ chains, are not hypervariable in these V_L regions.

At present, at least three different L_I regions can be distinguished among *b4* chains from A-variant polysaccharide antibodies. They are partially identical in *b5* chains by sharing a recurrent Asn-Asn sequence in positions 31b–c. Seven V_L regions sharing an amino terminal Ile-Val-Met sequence have an identical sequence in positions 1–40, including the L_I region. Two of these antibodies are also identical in the L_{III} region while two additional L chains of this subgroup have different L_{IV} regions. One of these latter two L chains probably shares the L_{III} region with a V_L region of a different subgroup. The other four L_{III} regions, completely or partially sequenced, show variations that do not readily follow an overall pattern of similarity within antibody L chains of this specificity.

If one attempts to estimate the number of V-region germline genes from the currently, completely, or partially known 67 rabbit L chain sequences, it is concluded that the germline of this species contained a minimum of from 27 to 49 V_L genes.

We conclude that these sequence data provide evidence that selective breeding of rabbits enhances the probability to find identical V_L-region sequences, provided the L chains belong to the same subgroups. These data further support the concept that V_L regions of the rabbit are subject to inheritance. However, there remain apparent difficulties in fully understanding the mode of inheritance based on the rabbit V_L sequences known to date.

H- and L-chain recombination data obtained with 15 selected chains from eight antibodies also tend to support the expectation that the H chains isolated from some of these A-variant polysaccharide-specific antibodies are likely to share substantial sequence similarities. While heterologous H and L chains isolated from an immunoglobulin pool are unable to reconstitute a specific function in association with H or L chain from anti-A-variant polysaccharide antibodies, several heterologous chain recombinants of these latter antibodies are as efficient in antigen binding (Farr

test) as the homologous recombinant molecules. If this comparative analysis of 25 recombinant antibody molecules from L and H chains produced by closely related rabbits is related to the H chain– *i.e.*, the L chain varies– four of 20 recombinants regained a higher antigen-binding capacity than one of the homologous pairs. Conversely, if this comparison is related to recombinants containing the same L chain, seven of 20 recombinants are higher in antigen-binding capacity than one of the homologous pairs. In general terms, these recombination data fully support the random pairing model of H and L chains describing the mechanism by which functional variable domains are assembled in the lymphocyte. Thus, if a genetically defined population of animals is selected as antibody producers to an antigen satisfying the qualification of epitope homogeneity, or at least a high degree of epitope restriction, heterologous, antibody recombinants are obtained that regain even better antigen-binding capacities than one of the homologous pairs. This suggests that the individual either fails to express the best possible pair in a single molecule or the genes for the polypeptide chains that could form this pair segregate, and are thus present in some and lacking in other individuals.

Sequence Diversity Among Heavy- and Light-Chain Variable Regions of Rabbit Antibodies Elicited by the Same Antigen. M.N. Margolies, L.E. Cannon, and E. Haber. Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts.

We previously reported the V_L amino acid sequence of three antibodies (#3368, #3374, #3381) to type III pneumococcal polysaccharide (S3). When these were compared to three other V_L sequences reported by Jaton, a different sequence in the hypervariable regions was found in each L chain. Three additional V_L sequences from S3 antibodies are reported here (3T70, 3T72, 3T74), as well as the partial sequence of three V_H regions of S3 antibodies carrying the a1 allotype (#3374, #3381, #3T72). The V_L sequences were obtained by automated Edman degradation of the intact chain and of tryptic peptides from citraconylated chains. The V_H sequences were obtained by automated Edman degradation of CNBr peptides and of tryptic peptides from citraconylated chains.

Comparison among these nine available V_L sequences for S3 antibodies reveals that no two sequences are identical in amino acid substitutions in their hypervariable regions (homologous to the first and third hypervariable regions of human myeloma L chains) and that they also differ in chain length in these regions.

Comparison of the three V_H sequences from S3 antibodies with two others previously reported reveals that sequence diversity among antibodies made against the same simple antigen is not limited to the L chains. A hypervariable region, extending from position 50 to 60 homologous to the second hypervariable region of human myeloma proteins, can be identified in the H chains. None of the five S3 V_H sequences is identical in this region. Sequence variations, although less marked, are also seen in the region homologous to the first hypervariable region of human heavy chains. In the region (78–90– homologous to the "extra" hypervariable region described by Capra and Kehoe, significant sequence variation is not seen (within a given allotype). However, two of the a1 chains have a one-residue insertion in this region.

The V_H amino acid sequence outside the hypervariable regions (framework) is highly conserved within the a1 allotype, in contrast to the V_L framework where greater variation was found.

The results indicate that a large number of different complementarity-determining sequences may be found in light and heavy chains from antibodies of the same allotype, specific for the same antigen.

Thus, antigenic stimulation results in the expression of a wide diversity of combining site sequences which are not seen when antibodies are selected for sequence study on the basis of shared idiotypy. (Supported by a Grant-in-Aid from the American Heart Association and NIH Grant AI 04967.)

IV. Regulation of Antibody Structure

Structural and Idiotypic Characteristics of Murine Anti-α(1,3)-Dextran Antibodies. Daniel Hansburg, David Briles, and Joseph Davie. Washington University School of Medicine, St. Louis, Missouri.

BALB/c mice can be induced to generate large amounts of IgM and IgG antidextran antibodies by means of an immunization protocol involving administration of both B1355 dextran and an

α(1,3)-bearing *E. coli*. These antibodies have the same specificity, class, and idiotype distribution as those stimulated by dextran alone. It is clear that the antibodies possess idiotypic determinants present on MOPC-104 and J558, α(1,3)-binding myeloma proteins. By preparative isoelectric focusing, IgG anti-dextran antibodies have been separated into M104-like and J558-like populations which are distinct. In addition, it is clear that M104 determinants are shared by IgM and several isotypes of IgG. (Supported by grants from the Public Health Service and several tobacco companies.)

Nonallelic Behavior of Rabbit Group-A Allotypes. M. Mudgett, B.F. Fraser, and T.J. Kindt. The Rockefeller University, New York, New York 10021.

Group-a allotypes not detected by qualitative typing or anticipated from breeding data (latent allotypes) were detected at low levels in 50 percent of normal rabbit sera tested by a sensitive radioimmune inhibition assay. The ability to detect comparable levels of latent allotypes in rabbit sera was demonstrated by several different anti-group-a allotypic sera. Latent allotypes were detected in sera from rabbits with all possible group-a allotypes, and their occurrence in individual rabbits was transitory and sporadic. Both IgG fractions from nonimmune sera and antibody fractions from group-C streptococcal antisera were shown to contain molecules bearing the latent group-a allotypes. One antibody fraction (4136-C) was shown to be 13 to 15 percent in latent group a3 allotype by both inhibition and direct binding assays. The latent allotypes present in these fractions were demonstrated to be serologically identical to the group-a marker present on radiolabeled ^{125}I-IgG, ^{125}I-homogeneous antibodies, and in the case of a3, the V_H (a3) fragment from IgG (a gift of L.E. Mole). The results, when taken together, indicate that latent group-a allotypes are serologically identical to authentic a allotype, and suggest that the expression of rabbit group-a allotypes are under some form of regulatory control.

Uniformity in the Clonal Repertoire for the Immune Response to Phosphorylcholine in Mice. J.L. Claflin and S. Rudikoff. University of Michigan Medical School, Ann Arbor, Michigan 48109, and the National Cancer Institute, Bethesda, Maryland.

Mouse strains, regardless of their genetic background, produce antibodies to phosphorylcholine (PC) which possess a specificity and binding-site idiotype indistinguishable from those of H8, a PC-binding myeloma protein. Recent studies have shown that three or four additional antibodies to PC, undetected with previous plaquing procedures, are present in immune sera; three of them are similar to the PC-binding myeloma proteins M603, M167, and/or M511. By analytical isoelectric focusing, at least four different light (L) chains were observed in purified anti-PC antibodies of the 20 strains studied. Three of the L chains correspond to those of H8, M603, and M167-M511 (the latter two focus similarly), and were uniformly expressed in all strains except BALB/c, which produced primarily the H8 type. Idiotypic analyis of the antibodies, using antisera directed to binding and nonbinding site regions, identified the H8, M603, M167, and/or M511 idiotypes. Fractionation of anti-PC antibodies with immunoadsorbents prepared with anti-idiotypic antisera showed a clear correlation between binding-site specificity, idiotype, and L chain type. These data suggest that the clonal repertoire for PC in any mouse strain is a set of four or five clones, and that a similar set, having the same array of binding regions, is present in every other mouse strain.

Corroborative evidence is provided by N-terminal amino acid sequence analysis of anti-PC antibodies from A/J mice. Isoelectric focusing of A/J anti-PC revealed the H8, M603, and M511-M167 L chains. By quantitative idiotypic analysis, this antibody consists of 30 percent H8-like, 27 percent M603-like, and 38 percent M511-M167-like antibody. With the possible exception of position 23, the H chain of this antibody shows a single sequence in the first 36 residues which is identical to that of the H chain of the BALB/c PC-binding myelomas H8, M603, and M511; all three have the same amino acid sequence through the first hypervariable region but differ in the second and third. M167 is also identical to these proteins with the exception of a Val-Leu interchange at position 4. Val is not seen at this position in the A/J heavy chain. Analysis of the L chains reveals three sequences, in about equimolar amounts: one identical to H8, the second identical to M603, and the third identical to M167. Thus, A/J and BALB/c mice, which differ considerably in their genetic makeup—including the IgC_H complex locus, produce antibodies to at least one antigenic determinant that are remarkably similar, if not identical, to each other in their variable regions. (Supported by USPHS NIH grant #A112533.)

Strain Analysis of Murine Monoclonal Antiphosphorylcholine Antibody. P.J. Gearhart, N.H. Sigal, and N.R. Klinman. University of Pennsylvania, Philadelphia, Pennsylvania.

Utilizing the technique of in vitro stimulation of individual B cells in carrier-primed splenic fragment cultures, we have shown that a majority of the antiphosphorylcholine (PC) response in BALB/c mice is represented by B cells' yielding antibody with the TEPC 15 idiotype. Such antibody is completely reactive with anti-T15 sera, produced in A/He mice, as well as anti-T15 antibody, raised in rabbits, that differs from mouse anti-idiotype in that it is specifically prepared to react with the hapten binding site of the myeloma protein. Thus, in an assay detecting ^{125}I-labeled T15 binding to anti-T15 antibody-coated test tubes, monoclonal antibody inhibits as well as does the T15 myeloma protein on a weight basis. While antibodies in both preparations of anti-T15 are specifically reactive with T15 protein (*i.e.,* they do not react with the PC-binding myeloma proteins MOPC 167 or McPC 603), both preparations appear to be somewhat heterogeneous, since both express some crossreactivity with MOPC 511. Therefore, it may be anticipated that only portions of these anti-idiotypes may react with some monoclonal antibodies.

These sera have been used to analyze the repertoire specific for PC in BALB/c mice and other murine strains. The total anti-PC B-cell response varies among the nonimmune strains in that, while BALB/c has the highest frequency (22.7 precursors/10^6 B cells), AKR, A, and C3H have about half this frequency, and CBA mice have a very low frequency. In BALB/c mice, 77 percent of the response is represented by apparently one clonotype, which reacts equally well on a weight basis with anti-Fab, rabbit anti-T15, and mouse anti-T15 antibody. These clones also display a characteristic isoelectric focusing pattern. A significant minority of the clones do not have T15 antigenic determinants, and this group appears to be heterogeneous by differences in hapten-binding properties. One percent of the BALB/c clones reacts as well with rabbit anti-T15 as the T15 protein, but has no reactivity with mouse anti-T15, and another one percent reacts on a 1:1 weight basis with rabbit anti-T15, but has only partial reactivity with mouse anti-T15. It has been reported by others that the rabbit anti-T15 preparation is specific for the binding site of the molecule, whereas anti-T15 antibody raised in A/He mice recognizes different variable-region determinants. Reactivity with rabbit antiT15, but not mouse anti-T15, thus may indicate that these one to two percent of anti-PC antibodies share some variable-region antigenic determinants with T15, but not all.

Similar analyses carried out with other strains show that most primary precursor cells do not possess the T15 idiotype and also appear to be heterogeneous by hapten inhibition. A significant fraction of AKR, A, and C3H monoclonal antibodies react equally as well as the T15 myeloma protein with rabbit anti-T15. When these clones were carefully analyzed for mouse anti-T15 activity, which has been reported to be absent in strains other than BALB/c, an interesting pattern of crossreactivity was observed. A very low frequency of PC-specific monoclonal antibodies in A and C3H (less than 1 in 3×10^6 B cells) reacts partially with mouse anti-T15 antibodies. The extent of these crossreactivities is variable, but may serve as a means of clonal identification. In AKR mice, however, one-third of the PC-specific B cells produce antibody that reacts as well with mouse anti-T15 and rabbit anti-T15 as it does with anti-Fab. Thus, using these anti-idiotypic antisera, the AKR clones were indistinguishable from the major BALB/c clonotype.

Within the context of heterogeneous anti-idiotypic antisera, identification of structural similarities can be made only in an operational sense. Nevertheless, analysis by anti-idiotypic sera has served as a means of identifying minor clonotypes, clearly revealing a heterogeneous array of precursors. We have found that some BALB/c antibodies share combining site-related idiotypic specificities with T15 but differ in other variable-region determinants, and that idiotypic determinants were shared among antibodies of strains that are allotypically distinct.

The Expression of Unusual Variable Regions. R. Mage, J. Rejnek, C. Alexander, and G.O. Young-Cooper. NIAID, National Institutes of Health, Bethesda, Maryland 20014.

In 1974, Strosberg and coworkers (*J. Immunol. 113*:1313, 1974) reported that a rabbit of allotype a1a3b4b5 responded to hyperimmunization with a vaccine of *Micrococcus lysodeikticus* by production of antibodies of restricted heterogeneity. The rabbit's serum then reacted with anti-a1, anti-a2, anti-a3, anti-b4, anti-b5, and anti-b6 antisera. We attempted to duplicate this observation of apparently unusual phenotypic expression of latent allotypes by immunizing 30 rabbits of defined pedigree and various allotypes with the same vaccine by a similar protocol. We typed weekly bleedings by gel diffusion in agar containing two percent polyethylene glycol. Cellulose acetate

electrophoresis showed that most (28 of 30) of the rabbits developed elevated levels of serum IgG, usually by the fifth week of immunization. Although some elevations in clonal products, detected as typical groups of three or four closely spaced bands on isoelectric focusing (IEF), were observed, there were no responses of highly restricted heterogeneity. One of three a1a3b4b9 offspring of a homozygous a3b4 sire and a1b9 dam produced an unusual IgG in elevated concentrations by the fifth week of immunization. This IgG precipitated strongly in agar gels weith the anti-a2 antibodies produced by a3 rabbits but not by a1a3 or a1 rabbits. The appearance of this IgG correlated with the appearance of several clonal products on IEF. When ^{125}I-labeled a3 anti-a2 was reacted with the focused serum on an IEF plate and examined by radioautography, one group of three bands was detectably labeled. The component recognized by a3 anti-a2 may contain the product(s) of one or more variable-region genes normally expressed at low levels but present in the genome of many a1 rabbits. Normal sera of homozygous a1, but not homozygous a3, rabbits can deplete the a3 anti-a2 of this specific antibody.

Results similar in many respects to ours were independently obtained in the laboratory of Dr. R. Hamers by W. van der Loo and are reported in his D.Sc. thesis, Free University of Brussels. Moreover, we confirmed and extended one of van der Loo's observations by showing that the antibodies in a3 anti-a2 sera which react with our unusual IgG from an a1a3 rabbit also react strongly with the sera of jackrabbits, cottontails, and a snowshoe hare. These observations, and those of Knight and coworkers (*Nature 253*:216, 1975), who showed that some anti-a allotype antibodies react with determinants on human IgG, suggest that interpretations of the genetic significance of at least some apparent expressions of latent allotypes should be made with caution. The a allotypes represent groups of antigenic determinants expressed on different subpopulations of variable regions. There is no doubt that hyperimmunization can alter the relative levels of expression of such subpopulations and can result in production of relatively large amounts of Igs with unusual variable regions.

Genetics of the Lambda-1 Light Chain in Mice. W. Geckeler and M. Cohn. The Salk Institute for Biological Studies, San Diego, California.

By radioimmunoassay, the $\lambda-1$ light chain class accounts for about one percent of the total light chain population found on normal serum immunoglobulins in most inbred mouse strains. In SJL and BSVS mice, however, normal serum $\lambda-1$ is reduced twentyfold. This low $\lambda-1$ phenotype behaves as a mutually inherited genetic trait. Thus, the F_1 between a $\lambda-1$ normal-level strain (*e.g.*, BALB/c or C57/BL6) and a $\lambda-1$ low-level strain like SJL has about a twofold lower level of $\lambda-1$ than the normal parental strain. An analysis of the F_2 progeny from a BALB/c × SJL cross indicates that the control of $\lambda-1$ level in serum immunoglobulin is under the control of a single genetic locus unlinked to allotype or *H-2*.

In mice with the $v_H$dex gene (*e.g.*, BALB/c) α-1,3 dextran elicits antibody whose light chain is entirely $\lambda-1$. These mice are high responders to dextran. When the $v_H$dex gene is present in mice with the low $\lambda-1$ phenotype (SJA/9 mice or appropriate F_2s described above), the antibody to α-1,3 dextran again contains only $\lambda-1$ light chains. The magnitude of the response, however, is reduced about fiftyfold below that of a high-responder strain.

Isoelectric focusing of anti-α-1,3 $\lambda-1$ from the high and low responder phenotypes described above shows a single predominant band in each. Both bands have the same isoelectric point as the λ_0-1 chain, which recurs in the myeloma collection.

The gene locus responsible for low-serum $\lambda-1$ and low α-1,3 responsiveness in $\lambda-v_H$dex may be the $\lambda-1$ structural gene itself. In that case, the amino acid replacement(s) must not alter the net charge of the chain. Several myeloma λ chains with such replacements in complementarity-determining residues are known. Replacement(s) in a complementarity-determining region might produce the observed phenotype by affecting the affinity of the α-1,3 antibody as well as the ability of $\lambda-1$ to produce viable antibody activities with other v_Hs. Alternatively, a regulatory gene which acts specifically on the $\lambda-1$ structural gene may account for the observed phenotypes. An analysis of the SJL $\lambda-1$ sequence is presently in progress to decide between these alternatives. (This work was supported by National Institute of Allergy and Infectious Deseases Research Grant AI05875 and National Institute of Allergy and Infectious Deseases Training Grant AI00430.)

Thymus-Dependent and -Independent PC-Specific B Cells. José Quintáns and Humberto Cosenza. Basel Institute for Immunology, Basel, Switzerland.

The in vitro response of BALB/c spleen cells to pneumococcus R36A is thymus independent (Cosenza *et al. Eur. J. Immunol. 5*:343, 1975), and consists of small clones with a peak size of 10 PFC on days three and four of culture. There are 20 to 37 PC-specific precursor cells/10^6 cells induced by R36A. The in vitro response to PC-KLH requires the presence of carrier-primed, theta-bearing cells, and so is considered to be thymus-dependent. PC-specific precursors induced by PC-KLH are less frequent than those induced by R36A (6–16/10^6 spleen cells), although they give rise to larger clones of 50 PFC/each on day five of culture (J. Quintáns and H. Cosenza, *Eur. J. Immunol.*, in press). The antibody secreted after immunization with R36A or PC-KLH has the same avidity and >95 percent of the clones expressed the TEPC 15 idiotype as determined in a plaque-inhibition assay. When R36A and PC-KLH were added to the same cultures, a synergistic effect was seen, *i.e.*, the number of plaques obtained was greater than the sum of the response induced by each antigen. Additionally, under microculture conditions where only B cells were limiting, R36A plus PC-KLH induced a higher number of precursor cells than did either antigen on its own. We consider these findings as evidence that thymus-dependent and thymus-independent clones, although displaying identical V regions, are different cells.

Regulation by Autogenous Complementary Antibody. Donald A. Rowley and Heinz Köhler. La Rabida Institute, University of Chicago, Chicago, Illinois.

Conceptually, antibodies of complementary idiotypy have combining site structures which are directed against each other. Such complementary antibodies were induced in A/He mice by immunization with PC-containing antigens and by immunization with the PC-binding IgA myeloma protein TEPC-15. Both responses were monitored by enumerating plaque-forming cells and assaying serum antibody levels against the corresponding antigens. Mice immunized at least three times with TEPC-15 in adjuvants had markedly suppressed responses to subsequent immunization with PC; similarly, mice preimmunized multiple times with PC had suppressed responses to immunizations with T15. In contrast, mice immunized with T15 in the interval between primary and secondary immunizations with PC had undiminished PFC responses to both antigens, but had significantly decreased antibody titers to PC. Simultaneous responses were also found when immunizations with TEPC-15 were superimposed on weekly immunizations with PC; with this regime, immunization with TEPC-15 actually enhanced the PFC response to PC, but serum antibody titer to PC were significantly lower than for mice immunized with PC only. Presumably, levels of serum antibody to PC were lower, either because anti-PC antibody was complexed with the complementary antibody directed against TEPC-15, or because the antibody directed against TEPC-15 prevented synthesis and/or release of anti-PC antibody by cells in vivo. Clearly, an autogenous immune response can alter dramatically a complementary response, but the effects of many variables, such as the amounts and ratios of the classes of antibodies and the nature of the mechanisms of regulation, remain to be defined.

IgG-Bearing Cells Carry IgG Memory. Ko Okumura, Leonore A. Herzenberg, Michael H. Julius, and Leonard A. Herzenberg. Department of Genetics, Stanford University School of Medicine, Stanford, California 94305.

Memory B cells which give rise to IgG antibody-producing cells were generally assumed to be Ig-bearing cells. However, recent studies indicating that very few IgG-bearing cells exist in lymphoid tissue brought this assumption into question (*cf.* Vitetta and Uhr, *Science 189*:964, 1975)

In this study, we examined directly the question of whether IgG-bearing cells contain functional precursors of IgG antibody-producing cells. Using the adoptive secondary immune response to DNP-KLH, we demonstrated that Ig-1b-bearing cells, isolated with the fluorescence-activated cell sorter (FACS) and then supplemented with KLH-primed T cells, are the functional precursors of Ig-1b-producing cells. Similarly, we showed that FACS-isolated IgG_2-bearing cells are the functional precursors of the IgG_2 antibody-producing cells and that isolated IgG_1-bearing cells are the functional precursors of IgG_1 antibody-producing cells.

Thus we have re-established the conventional wisdom concerning IgG memory cells. Whether IgD is also present on these cells remains to be seen.

Genetic Control of T-Dependent Antibody Response Defects in the BSVS Mouse. David Briles, Richard Krause, and Joseph Davie. Washington University School of Medicine, St. Louis, Missouri, and the National Institutes of Health, Bethesda, Maryland.

BSVS mice are defective in their immune responses to two thymus (T)-dependent antigens, DNP-hemocyanin (H) and streptococcal Group A carbohydrate (GAC), yet respond normally to the T-independent antigens, DNP-ficoll and pneumococcal carbohydrate (phosphorylcholine). In addition, the BSVS responses to DNP-H and GAC are indistinguishable from the responses of thymectomized (Tx) responder mice. Thus, BSVS and Tx-AKR are deficient in the IgG, but not the IgM response to DNP-H. On the other hand, both the IgM and IgG responses to GAC are depressed in BSVS and Tx-AKR mice. The immune deficit of the BSVS mice was studied in crosses with strain A mice. Hybrid (BSVS × A) mice were responders to both antigens. In the backcross (BSVS × A) × BSVS mice, there were equal numbers of anti-GAC responder and nonresponder mice, indicating genetic control by a small number of major loci. The anti-GAC responses of the backcross mice showed no obligate linkage between responder status and strain A *H-2* or IgC_H alleles. However, it was observed that the average anti-GAC titers were higher in backcross mice heterozygous at these loci. Taking into consideration these data, a lack of low-responder F_2 animals, it seems likely that the defect in the BSVS T-dependent anti-GAC responsiveness involves three loci, probably including *H-2* and IgC_H. (Supported by grants from the Public Health Service and several tobacco companies.)

Genetic Control of Susceptibility to Abelson Murine Leukemia Virus. Rex Risser, Michael Potter, and Wallace P. Rowe. National Institute of Allergy and Infectious Diseases, and National Cancer Institute, NIH, Bethesda, Maryland.

Abelson murine leukemia virus (MuLV-A) induces tumors of primarily B-cell origin in mice. The virus consists of helper leukemia virus and defective transforming virus, the latter being responsible for lymphoma induction. The frequency of lymphoma induction varies among inbred mouse strains, and thus the genetic control of susceptibility to MuLV-A can be investigated.

Intravenous inoculation of MuLV-A into one-week- to $3^1/_2$-month-old BALB/c mice results in 95 to 100 percent lymphoma induction with a mean latent period of 30 days. In contrast, C57BL/6 mice change from being highly susceptible to MuLV-A lymphoma induction at one week of age to being highly resistant at $1^1/_2$ to two months of age. Using two-month-old mice, we have investigated the pattern of susceptibility to MuLV-A lymphoma induction in (BALB/c × C57BL) F_1 mice and Bailey CXB recombinant inbred (RI) mouse strains. MuLV-A induces 70 to 80 percent incidence of lymphomas in the F_1 mice with an average latent period of 40 days. Four phenotypic patterns of susceptibility have emerged among the RI strains: CXB-D and -H resemble BALB/c; CXB-G and -I resemble C57BL; CXB-E and -K show a weak susceptibility; and CXB-J shows intermediate susceptibility. BALB.B mice, carrying the *H-2* region of C57BL, are as susceptible to MuLV-A lymphoma induction as are BALB/c. There is no correlation between susceptibility to MuLV-A and the inheritance of genes from BALB/c at Gpd-1(Fv-1), Fv-2, and IgC_H loci among RI mouse strains, nor does the pattern of susceptibility to pristane induction of plasmacytomas correspond to the MuLV-A susceptibility pattern among RI strains.

Possible in vivo restrictions on MuLV-A have been investigated by determining the fate of virus in target tissues following infection of susceptible and resistant mice. Both helper and transforming viruses are detectable at ten-day post-injection in BALB/c mice and increase in titer throughtout the latent period for lymphomagenesis. In contrast, in C57BL mice the transforming virus cannot be recovered during the same period, although helper leukemia virus is recovered in amounts comparable to BALB/c. The simplest interpretation of these results is that C57BL mice carry a major recessive gene which confers resistance to MuLV-A lymphoma induction, and that there are probably one or more minor genes for resistance as well. This resistance appears to be directed specifically against the defective transforming virus or cells producing it.

Plasmacytomas of NZB Mice. H.C. Morse III, R. Asofsky, R. Riblet, and M. Weigert. National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, and The Institute for Cancer Research, Fox Chase, Philadelphia, Pensylvania.

Plasmacytomas (PCT) were induced in NZB mice of both sexes by three intraperitoneal injections of Pristane given at monthly intervals. The incidence and time course of PCT development were identical to those previously observed in studies of PCT in BALB/c (C) mice. The frequency of tumors produced in male and female mice was the same. The heavy-chain class distribution of 230 primary NZB PCT secretory products, as determined by immunoelectrophoresis, differed strikingly from that previously described for 558 primary C PCT: γA-20.9 percent NZB, 45.5

percent C; γM-1.7 percent NZB, 1.1 percent C; γ1–9.1 percent NZB, 6.4 percent C; γ2a–15.2 percent NZB, 6.4 percent C; γ2b–25.7 percent NZB; 8.1 percent C; γ3–2.2 percent NZB, 0.7 percent C; free light chain: 1.7 percent NZB, 2.2 percent C. More than one paraprotein was detected in primary ascites of 9.6 percent of NZB and 6.6 percent of C PCT. Two tumors with antigen-binding capacity for α-1,6 dextran and one binding levan were γA producers.

The Stability of a Mouse Myeloma Producing Two Classes of Immunoglobulin: In Vitro Studies. M.E. Neiders, H.C. Morse III, A.R. Lawton III, M. Potter, and R. Asofsky. National Institute of Allergy and Infectious Diseases, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, and University of Alabama School of Medicine, Birmingham, Alabama.

The present studies were undertaken to determine the feasibility of establishing in culture a mouse myeloma that produces two classes of immunoglobulin, and to characterize the immunoglobulin products of the cultured cells. SAMM 368, a pristane-induced BALB/c myeloma that produces IgG2b (γH) and IgA (γA), was used for this study. Myeloma cells from ascites were adapted to culture from generations three to eight using several techniques, including alternating passage from culture to mouse (Periman, *J. Natl. Cancer Inst. 46*:403, 1971). From these attempts, five separate lines have been maintained in continuous culture for more than six months in RPMI 1640 medium supplemented with 20 percent inactivated horse serum, 4 mM glutamine, nonessential amino acids, penicillin, streptomycin and 5×10^{-5} M 2-mercaptoethanol.

Immunoglobulin production was monitored by three methods: 1. reinjection of the cells in mice and testing the resulting ascites fluid or sera with class-specific antisera using immunoelectrophoresis; 2. staining of tissue culture cells with rhodamine- or fluorescein-labeled purified antibodies; 3. autoradiography of immunoelectrophoretic patterns of supernatants from tissue culture cells cultured in vitro with medium containing C^{14}-labeled L-lysine and L-isoleucine (Hochwald *et al. J. Exp. Med. 114*:459, 1961). Up to three months in culture, all lines produced both immunoglobulins. At six months, one line produced only IgA, one line produced only IgG2b, while three lines produced both immunoglobulins. Reactions with monospecific anti-IgG1 (γF), anti-IgG2a (γG), and anti-IgM (γM) were not noted at any time the lines were examined.

Cloning of cells was undertaken to determine the regularity of maintaining the production of both immunoglobulins by single cells. For the pilot studies, a line that has been a consistent producer of both IgA and IgG2b when examined 11 times over seven months was in its sixtieth passage in vitro. Cytoplasmic staining, using double labeling with fluorescein- and rhodamine-labeled antibodies, revealed that 98.4 percent of the cells stained both with anti-IgA and anti-IgG2, while 1.3 percent stained only with anti-IgG2, and 1.3 percent stained only with anti-IgA. Cells were cloned in 0.3 percent agarose, as described by Coffino and coworkers (*J. Cell. Phys. 79*:429, 1972). Oil-induced peritoneal macrophages or spleen macrophages from syngeneic mice were used as feeder layers. Twelve days after cloning, randomly selected individual clones were removed and placed in 16-mm dishes. When cells had multiplied to cover the bottom of the dish, they were transferred to 60-mm dishes. Cells were transferred to additional dishes until the total of each clone reached $2–3 \times 10^6$ cells. At this time, cells from each clone were pooled and cultured in the presence of radioactive amino acids. A total of 20 clones were examined by autoradiography following immunoelectrophoresis of cell supernatants with BALB/c hyperimmune serum or SAMM 368 ascites fluid to assure sharp precipitin lines. The C^{14}-labeled supernatants of all 20 clones precipitated with anti-IgA antisera. All 20 clones also produced IgG2b.

These studies demonstrate that a myeloma producing two classes of immunoglobulin maintains the production of both immunoglobulins in vitro, but may give rise to lines that produce a single myeloma protein.

Plasmacytomas Producing Two Immunoglobulin Heavy-Chain Classes. H.C. Morse III, J.G. Humphrey, R. Lieberman, M. Neiders, A.R. Lawton III, R. Asofsky, and M. Potter. National Institute of Allergy and Infectious Diseases, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, and University of Alabama School of Medicine, Birmingham, Alabama.

Two plasmacytomas (PCT) were shown to produce two paraproteins—SAMM 368 and TEPC 609—over many transplant generations. SAMM 368 produced γ2b-κ and γA-κ, whereas TEPC 609 produced γ2b-λ and γA-λ. The γ2b-κ of SAMM 368, defined by class-specific heterologous antisera, does not carry γ2b allotypic markers and neither antiallotype or anti-idiotype could be raised by immunization of appropriate mouse strains. Idiotypic antisera raised in rabbits to

SAMM 368 γ2b did not crossreact with SAMM 368 γA. The γA of SAMM 368 bears appropriate allotype markers, and idiotypic antisera raised in mice did not crossreact with the γ2b of SAMM 368. Idiotypic antisera of mice produced against the γ2b or γA of TEPC 609 indicate that these paraproteins share idiotype. Immunofluorescent studies of ascites cells from SAMM 368 and TEPC 609 with rhodamine- and fluorescein-conjugated class-specific antiglobulins, purified by affinity chromatography, showed that for both tumors, single cells produce two heavy-chain classes.

Genetic Control of the Response to α1,3 Dextran. Roy Riblet, Joselina Gatmaitan, and Martin Weigert. The Institute for Cancer Research, Fox Chase, Philadelphia, Pensylvania.

The antibody response to α1,3 glucosyl linkages is controlled by an allotype-linked, autosomal dominant gene. For example, of 100 progeny of the backcross (BALB/c $\times$ NZB)$F_1 \times$ NZB, 48 mice expressed the BALB allotype and showed the high, idiotype-positive response characteristic of BALB/c and F_1 mice, and 52 expressed only the NZB allotype and showed the low, idiotype-negative response characteristic of NZB. Idiotype-positive response is defined as antidextran antibody which cross-reacts with the idiotype of J558 antidextran myeloma protein. The J558 idiotypic determinant results from a particular V_L (in this case V_λ) and V_H association, and idiotype-positive response is thus controlled by V_λ and V_H genes. An idiotype-negative response could be caused by differences in V_H and/or V_L genes. The allotype (C_H)-linked locus studied in the backcross is probably V_H, but if V_λ is linked to C_H this response gene could be V_λ. This ambiguity has now been resolved and V_λ is excluded.

We have induced λ chain-producing plasmacytomas in NZB mice and have obtained partial amino acid sequence data on two NZB λ chains to compare their structure with BALB/c λ from J558. Peptide compositions are known for the sequence from positions 22 to 75. The first two hypervariable regions are located in this region. The primary sequence has been determined for the region 89 to 105, which contains the third hypervariable region. These data indicate that the sequence of one protein, NZBPC2797, is identical to the J558 λ; while the other, NZBPC5060, has a single SER to ASN substitution at position 95. Thus, NZB mice have λ chains with the same combining site structure as those of BALB/c λ chains. Therefore, the inability of NZB mice to express antidextran antibodies with the 558 idiotype is the result of heavy-chain structural differences.

To accurately define the recombination frequency between V_H-DEX and C_H and to accumulate a panel of recombinant chromosomes for gene ordering, we have analyzed a total of 3091 backcross mice or their equivalent and have found 17 mice with a recombinant phenotype. Of these, 11 have so far been confirmed by progeny testing, yielding a tentative map distance of 0.36 cM. (Inbreeding of recombinant mice to obtain these chromosomes in a homozygous state for the ordering of V_H genes is in progress.)

These V_HDEX$-C_H$ recombinants have been tested for recombination in the region C_H-Pre. An unexpectedly high rate of multiple crossingover has been observed: in a series of six confirmed recombinants obtained in the cross of (BALB/c $\times$ A/He)$F_1 \times$ A/He, four chromosomes incurred a single crossover between V and C, with a gene order of V-C-Pre, while the other two require double crossovers, one between V and C and a second between C and Pre. Additional instances of multiple recombination have been noted in other crosses. This phenomenon may have significance for mechanisms of V-C translocation and allelic exclusion.

References

Barstad, P., Farnsworth, V., Weigert, M., Cohn, M., and Hood, L.: Mouse immunoglobulin heavy chains are coded by multiple germ line variable region genes. *Proc. Natl. Acad. Sci. U.S.A. 71*:4096–4100, 1974

Capra, J.D., Tung, A.S., and Nisonoff, A.: Structural studies on induced antibodies with defined idiotypic specificities. I. The heavy chains of anti-p-azophenyl-arsonate antibodies from A/J mice bearing a cross-reactive idiotype. *J. Immunol. 114*:1548–1553, 1975

Carson, D. and Weigert, M.: Immunochemical analysis of the crossreacting idiotypes of mouse myeloma proteins with anti-dextran activity and normal anti-dextran antibody. *Proc. Natl. Acad. Sci. U.S.A. 70*:235–239, 1973

Claflin, J.L. and Davie, J.M.: Clonal nature of the immune response to phosphorylcholine. IV. Idio-

typic uniformity of binding site-associated antigenic determinants among mouse antiphosphorylcholine antibodies. *J. Exp. Med. 140*:673–686, 1974

Cohn, M., Blomberg, B., Geckeler, W., Raschke, W., Riblet, R., and Weigert, M.: First order considerations in analyzing the generator of diversity. *In* E.E. Sercarz, A.R. Williamson, and C.F. Fox (eds.): *The Immune System: Genes, Receptors, Signals.* pp. 89–117, Academic Press, New York and London, 1974

Edelman, G.M. and Gottlieb, P.D.: A genetic marker in the variable region of light chains of mouse immunoglobulins. *Proc. Natl. Acad. Sci. U.S.A. 67*:1192–1199, 1970

Eichmann, K.: Idiotypic identity of antibodies to streptococcal carbohydrate in inbred mice. *Eur. J. Immunol. 2*:301–307, 1972

Eichmann, K.: Genetic control of antibody specificity in the mouse. *Immunogenetics 2*:491–506, 1975

Eichmann, K. and Berek, C.: Mendelian segregation of a mouse antibody idiotype. *Eur. J. Immunol. 3*:599–601, 1973

Ein, D.: Nonallelic behavior of the oz groups in human lambda immunoglobulin chains. *Proc. Natl. Acad. Sci. U.S.A. 60*:982–985, 1968

Farnsworth, V., Goodfleish, R., Rodkey, S., and Hood, L.: Immunoglobulin allotypes of rabbit kappa chains: polymorphism of a control mechanism regulating closely linked duplicated genes. *Proc. Natl. Acad. Sci. U.S.A. 73*:1293–1296, 1976

Gibson, D.: Genetic polymorphism of mouse immunoglobulin light chains revealed by isoelectric focusing. *J. Exp. Med. 144*:298–303, 1976

Gottlieb, P.: Genetic correlation of a mouse light chain variable region marker with a thymocyte surface antigen. *J. Exp. Med. 140*:1432–1436, 1974

Gutman, G.A., Loh, E., and Hood, L.: Structure and regulation of immunoglobulins: kappa allotypes in the rat have multiple amino-acid differences in the constant region. *Proc. Natl. Acad. Sci. U.S.A. 72*:5046–5050, 1975

Hamers-Casterman, C. and Hamers, R.: A second crossingover between d and a locus in rabbit immunoglobulin γ chain. *Immunogenetics 2*:597–603, 1975

Herzenberg, L.A., McDevitt, H.O., and Herzenberg, L.A.: Genetics of antibodies. *Ann. Rev. Genet. 2*:209–244, 1968

Imanishi, T. and Mäkelä, O.: Inheritance of antibody specificity. I. Anti-(4-hydroxy-3-nitrophenyl)-acetyl of mouse primary response. *J. Exp. Med. 140*:1498–1510, 1974

Imanishi, T. and Mäkelä, O.: Inheritance of antibody specificity. II. Anti-(4-hydroxy-5-broma-3-nitrophenyl)-acetyl in the mouse. *J. Exp. Med. 141*:840–856, 1975

Lieberman, R., Potter, M., Mushinski, E.B., Humphrey, J.R.W., and Rudikoff, S.: Genetics of a new Ig V_H (T15 idiotype) marker in the mouse regulating natural antibody to phosphorylcholine. *J. Exp. Med. 139*:983–1001, 1974

Lieberman, R., Potter, M., Humphrey, W., Jr., Mushinski, E.B., and Vrana, M.: Multiple individual and cross-specific idiotypes on 13 levan-binding myeloma proteins of BALB/c mice. *J. Exp. Med. 142*:106–119, 1975

Lundblad, A., Steller, R., Kabat, E.A., Weigert, M., and Cohn, M.: Immunochemical studies on mouse myeloma proteins with specificity for dextran or for levan. *Immunochemistry 9*:535–544, 1972

Mage, R., Lieberman, R., Potter, M., and Terry, W.D.: Immunoglobulin Allotypes. *In* M. Sela (ed.): *The Antigens,* Vol. 1. pp. 299–326, Academic Press, New York, 1973

Mäkelä, O., Julin, M., and Becker, M.: Inheritance of antibody specificity. III. A new V_H gene controls fine-specificity of anti-p-azobenzenearsonate coupled to the carbon atom 5 of hydroxyphenyl acetic acid in the mouse. *J. Exp. Med. 143*:316–328, 1976

McCarthy, M.M. and Dutton, R.W.: The humoral response of mouse spleen cells to two types of sheep erythrocytes I, II, and III. *J. Immunol. 115*:1316–1329, 1975

Pawlak, L.L., Mushinski, E.B., Nisonoff, A., and Potter, M.: Evidence for the linkage of the IgC_H locus to a gene controlling the idiotypic specificity of anti-p-azophenyl-arsonate antibodies in strain A mice. *J. Exp. Med. 137*:22–31, 1973

Potter, M. and Lieberman, R.: Genetics of immunoglobulins in mice. *Adv. Immunol. 7*:91–145, 1967

Potter, M. and Lieberman, R.: Common individual antigenic determinants in five of eight BALB/c IgA myeloma proteins that bind phosphorylcholine. *J. Exp. Med. 132*:737–751, 1970

Riblet, R., Weigert, M., and Mäkelä, O.: Genetics of mouse antibodies. II. Recombination between V_H genes and allotype. *Eur. J. Immunol. 5*:778–781, 1975

Spring, S.B. and Nisonoff, A.: Allotypic markers on F_{ab} fragments of mouse immunoglobulin. *J. Immunol. 113*:470–478, 1974

Steinberg, A.G., Milstein, C.P., McLaughlin, C.L., and Solomon, A.: Structural studies of an Inv (1,-2) kappa light chain. *Immunogenetics 1*:108–117, 1974

Strosberg, A.D., Hamers-Casterman, C., van der Loo, W., and Hamers, R.: A rabbit with the allotypic phenotype: a1a2a3b4b5b6. *J. Immunol. 113*:1313–1318, 1974

Taylor, B.A., Bailey, D.W., Cherry, M., Riblet, R., and Weigert, M.: Genes for immunoglobulin heavy chain and serum prealbumin protein are linked in mouse. *Nature 256*:644–646, 1975

Received November 8, 1976