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Meeting Report and Summary

Antibody Variable-Region Genetics:

Summary and Abstracts of the Homogeneous Immunoglobulin Workshop VII*

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The theme of this year's workshop was the genetic control of variable-region markers (V_H and V_L) with emphasis on studies in the mouse. Past workshops summarized in this journal (*Immunogenetics 1:*511–523, 1975 and *Immunogenetics 4:*401–435, 1977) have concentrated on descriptions of phenotypic differences in antibodies of the same specificity raised in different inbred mouse strains. For the most part, these differences are determined by antigenic specificities assigned to the V regions of immunoglobulins called idiotypes, though physicochemical (electrophoretic) differences, binding specificity, and primary sequence differences have also been exploited. It has been assumed plausibly that these kinds of markers reflect a difference in V-region structural genes. Based on this working hypothesis, analysis of different inbred strains for various idiotypes have partially delineated the V-region composition of a number of strains (V-region allogroups). Crosses between strains of different heavy-chain allogroups have occasionally yielded intraallogroup recombinants that order certain V_H genes with respect to each other and with respect to C_H. The picture of the heavy-chain locus that emerges from these studies is of two gene clusters (V_H and C_H) closely linked to each other with V_H being considerably larger than C_H. Though these studies are being actively continued with respect to both identification of new V_H-region markers and V_H linkage studies (see, for example, abstracts by Rosenstein, Pisetsky, and Riblet), this year a variety of issues relating to the nature of idiotypes and of their genetic control were raised. The possibility that some allotype-linked genes governing idiotypes were not V_H structural genes received support, so what have been considered V genes may include both structural and regulatory genes.

The Nature of Idiotype

Throughout the meeting there was considerable discussion of idiotypes. This term has different usages, depending upon the laboratory. To those outside

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the immunological field, the term often eludes definition because there are so many interpretations of what an idiotype is. It would seem clear that idiotype is an antigenic determinant that is determined by V domains in the immunoglobulin molecule. Since a V domain is a relatively large structure, it can be the source of more than one idiotype. There are two general descriptive characteristics of an idiotype. The first is *topographical*: a determinant associated with the framework of V_L or V_H; the complementarity determining region (CDR) of V_L or V_H domain; or a structure such as the antigen-binding site, resulting from the interaction of V_L and V_H. The second is *systematic* and is based upon the distribution of the idiotype on related myeloma proteins or homogeneous antibodies. Relationships may be determined by the presence of common V_L and V_H, and the presence of a common V_L or V_H. Idiotypes unique to a single homogeneous antibody of myeloma protein are individual, IdI. Idiotypes shared by different antibodies are referred to as IdX (cross-specific). IdX determinants of mouse antibodies can vary in degree of cross-specificity, ranging from IdX on antibodies with related antigen-binding properties to IdX on antibodies of unrelated specificities such as the 173:U10 idiotype described by Bosma and coworkers (1977), which resembles more the "a" allotypes of the rabbit.

The topography of IdX determinants restricted to myeloma proteins and antibodies that bind the same antigen is currently being studied. Lieberman and coworkers (these abstracts) have demonstrated in the inulin system that some IdX could be assigned to the V_H and others to the V_L. None of the IdX antisera were able to bind separated chains nor the chains recombined with a noninulin-binding protein. Thus, these markers require the cooperation of both V_L and V_H for expression, even though they are topographically assigned to a given V region. Vrana and coworkers (these abstracts) have attempted to determine the structural basis of an inulin IdX (IdX-B), using comparative amino acid sequence data and a hypothetical model of an inulin-binding myeloma protein. He was able to demonstrate that IdX-B was determined by two unique amino acid replacements appearing in L3 and L1 CDR which converge to form a continuous structure on the antigen-binding surface.

The most widely studied idiotype is the T15 idiotype associated with PC binding myelomas and antibodies. There is general agreement that several T15 idiotypes can be defined by subspecificities: T15-M511 IdX (binding site), T15-M511 IdX (nonbinding site-related), T15 IdI (binding site-related), and T15 IdI (nonbinding site-related). It should be pointed out that T15 V_L and M511 V_L are derived from very different V-kappa isotypes, so that certain sera may, in fact, be V_H-specific. It is not clear that all genetic studies using T15 idiotypes are the same. A more systematic study using these various sera would be useful.

Allotype-linked genetic control of IdX has been established for several examples, but the level at which IdI determinants are controlled is not clear. Mushinski and coworkers (these abstracts) were able to find the IdX shared by seven β1,6 galactan-binding myeloma proteins on induced antigalactan antibody. However, only one of the seven IdI determinants could be detected. In a previous report (Lieberman *et al.* 1976), Lieberman and coworkers were unable to find myeloma protein-associated IdI determinants on anti-inulin antibodies. Williams and Claflin (these abstracts) have not detected the IdI associated with phospho-

choline (PC)-binding myeloma protein, MOPC603, in anti-PC antibody. On the other hand, Hansburg and coworkers (these abstracts) have been able to dissect anti-α 1,3 dextran antibody into multiple species by isoelectric focusing. Among these species, three were shown to possess myeloma-specific IdIs.

The difference in expression of IdIs in these cases may reflect differences in the sensitivity of the assays used. The amounts of induced anti-inulin, antigalactan, and anti-PC antibodies are generally low, and IdI determinants in antiinulin or antigalactan antibodies are measured by hemagglutination-inhibition (HI) tests. In contrast, the amounts of antidextran antibody are considerably greater and IdIs are detected by a radioimmunoassay. It is thus conceivable that IdI determinants may occur among subpopulations of IdX-bearing anti-inulin and antigalactan antibodies, but are not revealed by the assays used. Alternatively, the IdI determinants of the inulin- or galactan-binding myeloma proteins may be the result of somatic mutation, as is suggested by various authors (these abstracts). By this view, an antibody is under germ-line genetic control that bears IdX determinants. Somatic mutations of this germline gene can alter certain IdX determinants, resulting in a collection of antibodies with unique determinants (IdIs), as well as determinants coded for by unmutated portions of the gene. Since these mutations are likely to occur at random, it is unlikely that a given IdI would occur reproducibly, thereby explaining the inability to detect IdIs in induced antibody. A precedent for this sort of mechanism can be found in the studies on somatic variants of the PC-binding myeloma protein S107, certain of which have altered PC-binding properties and can be presumed to have different IdIs (Cook and Sharff, these abstracts).

A somatic mutation basis for IdIs seems unlikely for the antidextran system, for which various IdIs can be reproducibly elicited (Hansburg *et al.*, these abstracts). A more plausible explanation for this case is that certain strains inherit a set of related V_H germline genes. The products of this related set, in combination with λ chain, share a determinant(s), IdX; but each product is unique as judged by different IdIs. The genetic implication of this model (that may be generally applicable to other cases if failure to detect IdIs results from technical reasons) is that strain-specific IdX differences are not under single, "V_H" gene control. Instead, strains must differ by "V_H" gene clusters, possibly a result of recent V_H gene duplications or deletions.

The Interpretation of Idiotype Differences

Idiotypes that would be most useful in genetic studies should be topographically assigned to a V_L or V_H isotype. Ideally, the different alleles of homologous V isotypes could be recognized individually. The presence of allelic idiotypes would obviate using such markers as "+" and "−" or high and low, which may indicate other factors interfering with the expression of the V-region genes. In this ideal situation there is assured penetrance of both alleles.

The present status of the available markers indicates that most do not fulfill these specific characteristics. Accordingly, interpretation of less than ideal markers may be fraught with some hazard. For example, the marker on phospho-

choline antibodies originally described (Lieberman *et al.* 1964) was low or absent in the prototype strain C57BL but easily demonstrable in BALB/c. Gearhart and Cebra (these abstracts) find that B lymphocytes from C57BL mice, stimulated in vitro, express the T15 Id; they point out that serum antibody levels do not necessarily reflect the genetic potential. Cancro and coworkers (these abstracts) extend this observation by finding a lack of concordance between the number of precursor B lymphocytes capable of producing T15 Id and serum levels of T15 Id in the Bailey RI strains. The serum levels of T15 Id, though, were similar to those previously reported by Lieberman and coworkers (1974), using the HI method. Thus, there is a strain-specific difference, controlled by one or more genes, that appears to regulate the levels of serum T15 Id. The intriguing property of this phenotype is that it is linked to the IgC (allotype) locus, yet cannot be a structural gene for the T15 Id, since both BALB/c and C57BL appear to have the genes to generate the same immunoglobulins.

A strong case for regulation of an antibody gene has been made for mouse λ chain. Earlier studies demonstrated a significant difference between SJL and other strains in the level of λ chain in normal immunoglobulin (Geckeler *et al.* 1976). By two approaches it has been shown that the level of λ chain is not the result of a structural gene defect in the λ^{10} strain SJL. As described by Geckeler and Cohn (these abstracts), no obvious structural differences can be detected between λ chains from λ^{10} (SJL) and λ^{+} (BALB/c) strains. A more decisive test of λ structural gene measures the capacity of the λ chain of the λ^{10} strain to function in antidextran antibodies. Since it is known that certain anti-α 1,3 dextran antibodies require λ chain to form the DEX^{+} idiotype (Carson and Weigert 1973), a λ^{10} strain can be tested for the ability to express this idiotype. Though this cannot be determined directly on SJL, because the heavy chain allogroup of this strain does not contain the appropriate V_H gene(s) for the expression of the DEX^{+} idiotype, this problem can be circumvented by identifying among the progeny of (BALB/c $\times$ SJL)F_2 examples that are λ^{10} with the BALB/c allotype. Dextran immunization of these examples elicited the DEX^{+} idiotype, indicating a functional λ chain. Lieberman and coworkers (these abstracts) have now described a λ chain allotype, and have shown that the λ^{10} phenotype segregates with the λ^{-} allotype. Hence, it appears that the λ regulatory gene and the λ structural gene are linked.

In view of these various findings, it is clear that the interpretation of an idiotype as a marker for a V-region structural gene must be made with caution. In certain instances, as for λ chain, linkage between the regulatory example and structural gene has been found. The studies suggesting regulatory control over the T15 idiotype would also imply a linkage between regulatory and structural genes insofar as the T15 regulation is linked to the C_H locus. (This argument is, of course, based on the assumption that heavy chain allotypes are controlled by structural genes—a concept shaken by the findings in the rabbit and mouse of hidden or latent V- and C-region allotypes; see review by Strosberg 1977.) The linkage studies using the λ^{10} marker and the T15 idiotype may, therefore, indicate the approximate location of antibody structural genes.

The relationship of regulatory genes linked to structural genes can conceivably take a variety of forms. The regulation could act on the entire V and/or

C locus, as in the case of mouse λ, and as would seem to be the case for allotype-linked control of immunoglobulin levels (Biozzi 1972). Regulation of specific V and C genes might also occur. If the T15 phenomena applies to other idiotypes, it should be recalled that recombination between genes controlling idiotype have been described (Weigert and Potter 1977). Therefore, certain of the putative V_H regulatory genes must be interspersed in the V_H locus.

In addition, there is a variety of ways by which antibody structural genes could be regulated by unlinked genes. As described below, two examples of V_L control over idiotype expression have been discovered (Imanishi-Kari *et al.* and Nisonoff *et al.*, these abstracts). Thus, the expression of an idiotype dependent on appropriate V_H and V_L genes could be controlled by either V_H or V_L. Another obvious way by which antibody genes can be regulated by loci unlinked to antibody structural genes is related to T-cell suppression of antibody production. A number of examples of specific idiotype expression mediated by T cells have been reported (Nisonoff, these abstracts). Cantor (these abstracts) has suggested a general T-cell suppression of "dominant idiotypes." In addition, T-cell suppression of allotype has been observed (Bosma *et al.*, these abstracts), which in turn could lead to suppression of allotype-linked V_H genes, the expression of which is dependent on adjacent C_H gene expression. It is, of course, conceivable that analogous regulation of V_L and C_L regulation may also occur. If the ability to suppress idiotype or allotype is under the control of separate immune response genes, expression of a given idiotype, sets of idiotypes, or allotypes may be unrelated to the genotype of the structural genes.

In view of the variety of ways that antibody expression might be regulated, our concepts of the organization, size, and location of heavy- and light-chain loci may need re-evaluation. As a first step, the location of structural genes needs to be unambiguously established. So far, by conventional genetic analysis only certain mouse V_κ loci have been assigned a chromosomal location (for recent studies on V_κ see abstracts by Gottlieb, Claflin, and Gibson). Particular caution must be taken in the interpretation of κ-chain genetics as monitored by the V_κ markers currently used. Since these are measured by semiquantitative assays, it is possible that low levels of a given V_κ gene product would be undetected. Strains for which particular V_κ structures are absent may be demonstrating the type of regulation observed in SJL of the λ structural gene.

Genetic analysis of the chromosomal location of antibody genes using what can be regarded as the best possible marker for structural genes, the mouse allotypes, have so far been unsuccessful. The earliest studies of this type were those of Potter and Lieberman (1968) and Herzenberg and coworkers (1968), who tested C_H linkage to a number of mouse genes. These studies and subsequent linkage studies carried out in various labs have failed uniformly to link the C_H genes to any of the genes tested. The simplest explanation for this failure is that the mouse chromosomes—for example, Ch 3 and 16—are not adequately saturated with useful markers. The finding of Taylor and colleagues of the serum prealbumin-C_H linkage (10–12 cm apart) offered some hope for refining the previous studies, *i.e.*, prealbumin might be closer to some of the markers previously tested. However, as described by Taylor and Eicher (these abstracts), the Pre-1 locus also appears not to be on any linkage group for the markers

tested in their survey. Again, as pointed out by Taylor and Eicher, this may reflect the inadequacies of detecting linkage for some of the markers used. Alternatively, the failure to find linkage of the H-chain locus might have to do with certain anomalies observed in the studies on three-factor crosses involving V_H genes and C_H or involving the complex V_H, C_H, Pre. As described here (Riblet, these abstracts), the frequency of double crossovers in this complex is significantly higher than expected. This finding may be related in some way to the unique nature of the H-chain locus, which involves a series of genes with a reasonably high degree of homology which might conceivably stimulate high frequencies of recombination that could obscure the linkage of the H-chain locus to other genes.

L-Chain Genetics

A feature of many of the idiotype markers described thus far has been that they are controlled by genes linked to the C_H locus. Assuming that both V_H and V_L contribute to the makeup of certain idiotypes, it would be expected that these would be controlled by both V_H and V_L genes. That most genetically controlled idiotypes are linked to allotype implies (barring linkage of light- and heavy-chain genes) that all mouse strains have functionally equivalent sets of V_L. Furthermore, as described below, the V_κ library appears to be extremely large. If V_κ were substantially larger than V_H the probability of finding an idiotype dependent on a particular V_L might be considerably lower than for V_H. In principle, L-chain control of idiotype expression should occur at least occasionally, and two cases have now been described. The Imanishi-Kari group (these abstracts) has shown that the absence of a particular idiotype on anti-NP antibodies in SJL mice is *not* caused by V_H, but by the λ^{10} phenotype of SJL. Nisonoff and coworkers (these abstracts) have demonstrated that in certain backcross segregants, the absence of an anti-AR idiotype segregates with the *Ly-3* locus.

The first of these experiments takes advantage of differences among strains in the level of λ-chain expression. As described by the Imanishi-Kari group (these abstracts), the finding that the anti-NP antibody of a particular idiotype usually associated with the Ig-1^b heavy-chain allogroup uses λ chains suggests a basis for the uncharacteristic response of the Ig-1^b strain, SJL. Based on the finding of the λ^{10} phenotype of SJL and on the knowledge that BALB/c can contribute an appropriate λ chain to this type of anti-NP antibody, the Ig-1^b allotype congenic CB-20 is idiotype-positive (Imanishi and Mäkelä 1975), (SJL $\times$ BALB/c)F_1 hybrid mice can be tested to locate the origin of the idiotype-negative response of SJL. The primary response in these hybrids is characteristic of the other Ig-1^b strains with respect to idiotype and fine specificity. It can be concluded that the idiotype-negative phenotype of SJL is caused by the λ^{10} phenotype of SJL, rather than by a gene(s) in the heavy-chain allogroup.

Nisonoff and coworkers (these abstracts), in a series of elegant experiments, reasoned that a possible source of idiotypes controlled by L-chain could be the mouse strains (AKR, C58, RF, and PL) known to have V_κ isotype differences

(Gottlieb, Claflin, and Gibson, these abstracts). To review briefly the system developed by Nisonoff and coworkers, A/J and AL/N mice respond to p-azophenylarsonate with a collection of antibodies (anti-Ar), most of which bear a characteristic idiotype. This idiotype is also expressed in the allotype-congenic strain C.AL-20, showing allotype linkage of the expression of the anti-Ar idiotype and suggesting that L chains of BALB/c are equivalent to AL/N in constructing the idiotypic determinant. This conclusion is reaffirmed by the results of the backcross $(A/J \times BALB/c)F_1 \times BALB/c$; complete concordance is found between the segregation of the A/J allotype and the anti-Ar idiotype. However, the backcross $(A/J \times PL/J)F_1 \times PL/J$ yields different results: only some of the segregants that have the A/J allotype express large amounts of the idiotype, and the rest express significantly lower amounts. The latter group were all shown to be homozygous for the Ly-3.1 phenotype of the PL/J strain. Thus, the locus responsible for the low expression of the idiotype is linked to *Ly-3*, as are the loci controlling the expression of V_κ isotypes.

It is important to keep in mind the nature of the V_κ polymorphism between PL/J and other strains, such as A/J. In part, it is based on the presence or absence of a particular V_κ isotype(s) typified by the Ib peptide (Gottlieb, these abstracts) or by differences in IF banding patterns of total kappa (Gibson, these abstracts). As is characteristic of all V-region markers so far, there is no firm evidence for allelic forms. Furthermore, the assays used to detect V_κ markers may be measuring quantitative differences in the expression of V_κ isotypes. It is conceivable that strains believed to lack an isotype may indeed be expressing low levels of the isotype, analogous, then, to the λ^{10} phenotype of SJL mice. The *Ly-3*-linked kappa locus may thus represent a regulatory gene controlling the expression of a subset of V_κ genes, which, in turn, may or may not be linked to the C_κ structural gene(s).

V-Region Diversity in the Mouse

A direct approach toward assessing the complexity of V-region loci comes from the comparison of V-region sequences of myeloma proteins or restricted antibodies. With the exception of λ_1 or λ_2, mouse myeloma proteins show considerable V_L- and V_H-region framework diversity. Tables 1 and 2 show a collection of V_H and V_L framework region sequences up to the first hypervariable region. These V_H and V_L framework regions have been divided into isotypes (also referred to as subgroups) on the basis of sequence homology. For the sake of convenience, a sequence differing by three or more residues from any other sequence in this part of the framework is designated as a separate isotype. By this arbitrary definition, 10 V_H and V_κ 26 isotypes have been identified among BALB/c myeloma proteins. It should be recognized that these values are minimum estimates: as more examples of myeloma proteins are sequenced, additional isotypes will be found. When examples of V_L or V_H are completely sequenced, additional framework differences should be found, as is already the case in the V_H-1 or V_κ-21 isotypes, for example.

The large number of V_H and V_κ isotypes already found among the BALB/c

Table 1. V_L Isotypes of the BALB/c Mouse[a]

Representative Myeloma Protein	VK	1	2	3	4	5	6	7	8	9
Prototype	VK_0	D	I	V	M	T	Q	S	P	A
MOPC460	VK_1		V					T		L
SAMM368G	VK_{26}		V	L				T		L
McPC843	VK_2		V					T		L
W3129	VK_3		V		V			T	G	L
S117	VK_4	E			L					
MOPC29	VK_5	E	N		L					
TEPC29	VK_6	E	V		L		Z			
LPC1	VK_7									S
McPC603	VK_8									S
										S
MOPC41	VK_9			Q						S
MOPC173	VK_{10}			Q				T	T	S
				Q				T	T	S
EPC109	VK_{11}			Q		I				S
MOPC31C	VK_{12}			Q						
				Q						
MOPC149	VK_{13}			Q					P	B
HOPC5	VK_{14}								T	K
MOPC21	VK_{15}	N								K
MOPC47A	VK_{16}								S	
									S	
MOPC157	VK_{17}								Q	S
McPC600	VK_{18}			Q		I	Z			S
MPC11	VK_{19}	N							H	K
McPC773	VK_{20}	E	T	T	V					
MOPC70E	VK_{21A}				I					
MOPC63	VK_{21B}									
TEPC124	VK_{21C}									
TEPC15	VK_{22}									T
MOPC46	VK_{23}				L					
MOPC511	VK_{24}				I			B	E	L
					I			B	E	L
SAMM368A	VK_{25}	E						A	A	F
	$V\lambda$	P	A	V	V	T	Q	Q	S	A
MOPC104E	$V\lambda 1$									
MOPC315	$V\lambda 2$						E	E		

[a] Modified from M. Potter: Antigen-binding myeloma proteins of the mouse. *Adv. Immunol.* *25*, in press, 1977. The V_L region of a number of myeloma L chains are classified according to their NH_2-C_{23} peptide sequence. An isotype is defined if the sequence in this region differs

myeloma proteins must indicate a high degree of complexity of the V_H and V_κ loci. Since most theories on antibody diversity agree that each different isotype must be coded for by a separate germline V gene, the sequence data suggest a minimum of 26 V_κ genes, and it has been predicted by statistical arguments (Cohn *et al.* 1974), that the V_κ locus contains at least 200 genes.

10	11	12	13	14	15	16	17	18	19	20	21	22	23
S	L	S	V	S	L	G	E	R	V	T	I	T	C
	T						D		A	S		S	
		P					D	Q	A	S		S	
T				T	I			P	A	S	L	S	
		P			M		D			S		S	
I	T	A	A				Q	K					
I	M		A		P						M		
I	M		A				L			S			
	M	Q	A		I			K				S	
					A			R			M	S	
					A						M	S	
			A							S	L		
			A				D						
			A				D					S	
			A				D	I			M		
			A		V								
					V			T					
					V								
Y			A		V			T					
F	M		T		V		()			S			
	M		M		V						L		
E					V		G						
()	L				V		G						
F	M		T		V		D			S	D		
	M	F	A		I		D	Z		S		S	
F	M		T		V		B			S		S	
			M	A	I			K					
		A					Q		A			S	
F		A		T	A	S	K	K				S	
T				T	P		D	S		S	L	S	
	D	P			S			S		S			
	K	P			S			S		S			
	N	P		T			T	S	A	S		S	
	L	T	T	S	P	G	E	T	V	T	L	T	C

from any other example by three or more amino acid replacements. A representative myeloma L chain for each V_L isotype is indicated; however, for most of these isotypes, more than one example is known

This complexity of V_κ and V_H regions is likely to be a feature of all mouse strains, since sequence companions of NZB myeloma proteins suggest that the number of V_κ and V_H isotypes will be as large as in BALB/c (Weigert and Riblet 1976.) The complexity of V_κ for a number of inbred strains is also indicated by the reproducible banding patterns obtained by isoelectric focusing

Table 2. V_H Isotypes of the BALB/c Mouse[a]

Representative Myeloma Protein	VH	1	2	3	4	5	6	7	8	9	10	11
Prototype	VH-0	E	V	K	L		E	S	G	G	G	L
S117	VH-1A					L						
SAPC10	VH-1B					L						
UPC10	VH-1C					L						
MOPC173	VH-1D					L					P	
SAPC15	VH-2			Q		V		T				
MOPC471	VH-3					V						
TEPC15	VH-4A					V						
W3207	VH-4B					V						
McPC	VH-4C					V						
MOPC511	VH-4D					V						
MOPC167	VH-4E				V	V						
W3082	VH-5					E					P	
MOPC21	VH-6	D	Q			V					P	
MOPC104E	VH-7		Q		Q	Q			P	E		
			Q		Q	Q			P	E		
MOPC315	VH-8	D		Q		Q				P		
MOPC460	VH-9			Q		Q				P	S	
S176	VH-10			Q		Q				T	V	

[a] (Modified from M. Potter: Antigen-binding myeloma proteins of the mouse. *Adv. Immunol.* 25, in press, 1977). The V_H regions of myeloma H chains are classified according to their amino terminal peptide residues 1–27. An isotype is defined if the sequence in this region differs from any other example by three or more amino acid replacements. A representative myeloma H chain for each V_H isotype is indicated; however, additional cases are known for most of these V_H isotypes

of pooled κ chain (Gibson, these abstracts). A variety of different κ bands (ca. 60) have been observed and it is possible that each band may be the equivalent of at least one V_κ isotype. It is also apparent that different inbred strains have V_κ isotypes in common; for example, identical V_κ isotypes have been found between NZB and BALB/c, and many of the IEF bands are shared between strains. Different strains, however, have certain unique isotypes, as indicated by the band differences observed by Gibson (these abstracts).

A central issue is the quality of these estimates of the upper limit of V_κ isotypes. Will further inspection of the framework of members of one isotype (beyond residue 23) reveal additional isotypic differences? Already the $V_\kappa 21$ isotype must be extended ($V_\kappa 21$A, B, C; Table 1). Will the pattern of variability within a V_κ isotype duplicate that found for V_λ? A similarity is certainly indicated from RNA/DNA hybridization studies indicating that the reiteration frequency of genes coding for V_λ and $V_\kappa 21$C (prototype myeloma L-chain, M321) are correspondingly low.

The question of how to evaluate isotypes is now being pursued by several groups. Considerable impetus to this analysis has come from the development of V_κ isotype antisera (McKean *et al.* 1973). Such antisera as used by Julius

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
V	Q	P	G	G	S	L	K	L	S	C	A	A	S	G	F
		L													
			K											—	—
	Z						R							T	
							R					T			
							R					T			
							R					T			
							R					T			
							R					T			
						M					V				
							R								
K			A		V		M			K				Y	
K			A		V		M	A		K		V		Y	
K			A		V		M	A		K		V		Y	
	K		S	Q			S		T		S	V	F		Y
	K		S	Z	T		S		T		S	V	T		S
A	R			S				M							

and coworkers (these abstracts) allow rapid screening of the BALB/c and NZB myeloma protein libraries to identify examples of a particular isotype. Furthermore, these antisera can be used to measure the degree of expression of V_κ isotypes in normal serum. This measurement permits a critical evaluation of the question of how representative the myeloma library is of normal immunoglobulin. As described here (Julius *et al.*), the frequency of a particular isotype among NZB and BALB/c myeloma proteins corresponds to the percentage of that isotype in the normal L-chain population. This result lends considerable confidence to the strategy of equating the results on diversity of myeloma V_κs to that of the normal V_κ population.

Identification of additional V_κ21 examples has allowed an extension of the earlier studies on diversity within the V_κ21 isotype (McKean and Potter, Hood *et al.*, Julius *et al.*, these abstracts). New V_κ21 examples in BALB/c and NZB continue to have identical or nearly identical amino terminal sequences through residue 23. (V_κ21B types such as M63 differ from V_κ21A and C by asparagine, instead of aspartic acid at residue one.) Additional BALB/c and NZB examples of V_κ21A, B, and C isotypes have been sequenced beyond residue 23, showing within the V_κ21B and the V_κ21C groups differences only in hypervariable regions analogous to the pattern of variability of V_λ. However, examples of minimal framework variation have been found for both the V_κ4 isotype (Rudikoff, these abstracts) and the V_κ21 isotype (Loh *et al.*, these abstracts). It is apparent that we do not know how many V_κ isotypes will ultimately be found in the mouse, and the need for further sequence data is obvious.

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I. Heavy Chain Genetics

Anti-Inulin (β-(1→2)-Linked Polyfructose) Responses in Inbred, Ig-Congenic, and Recombinant Inbred (RI) Mice. C.C. Chien, J.K. Inman, and R. Lieberman. Laboratory of Immunology, NIAID, NIH, Bethesda, Maryland 20014.

A previous report (R. Lieberman, M. Potter, W. Humphrey, Jr., and C.C. Chien: Idiotypes of inulin-binding antibodies and myeloma proteins controlled by genes linked to the allotype locus of the mouse. *J. Immunol. 117*:2105–2111, 1976) has indicated that an inulin cross-specific idiotypic determinant (InuIdX) was present in some strains (BALB/c) and absent in others (*e.g.*, C57BL). Studies with Ig-congenic strains. RI strains, and backcross progeny from BALB/c and C57BL have indicated that InuIdX is a V_H marker linked to the BALB/c allotype.

A problem encountered in this study was the low level of anti-inulin antibodies found in many strains following immunization with bacterial levan (a high molecular weight, β-(2→6)-linked, fructose polymer with β-(1→2) branching). Accordingly, we partially carboxymethylated inulin, prepared an active ester of the bound carboxyl groups with hydroxybenzotriazole, and coupled this reactive derivative to the amino groups of heat-killed bacteria (*S. tel aviv* and *B. abortus*).

The sera from mice immunized with these conjugated antigens were examined for anti-inulin and antilevan antibodies and for BALB/c InuIdX by the methods previously described (R. Lieber-

man, M. Potter, W. Humphrey, Jr., and C.C. Chien: Idiotypes of inulin-binding antibodies and myeloma proteins controlled by genes linked to the allotype locus of the mouse. *J. Immunol.* *117*:2105–2111, 1976).

BALB/c and RIII each responded well to the conjugated antigens (Inulin HA titers of 7–8 and levan titers of 8–9). C57BL were poor responders, and only 10 to 15 percent of 50 mice gave significant anti-inulin responses; all 50 mice had high levan HA activities (titers of 7–8). Individual NZB mice showed a wide range of variation in inulin and levan HA titers (1–10). Inulin antibodies were present in BALB/c, RIII, C57BL, and NZB, but only the first two strains showed the BALB/c InuIdX. Immunized RI strains and backcross progeny involving BALB/c and C57BL showed high anti-inulin responses in mice carrying the BALB/c allotype.

While most strains immunized with bacterial levan showed a moderate rise in anti-inulin levels with a correspondingly higher rise in antilevan titers, most strains immunized with bacteria-coupled inulin showed a moderate rise in antilevan titers and a much higher rise in anti-inulin levels.

Anti-inulin titers in Ig-1^a-congenic strains, BC-8 (congenic to C57BL) and SJA (congenic to SJL) were four to five, while BALB/c had anti-inulin titers of seven to eight. All of these mice showed the InuIdX marker. CB-20 (congenic to BALB/c, but carrying C57BL allotype) showed significant anti-inulin levels in 50 percent of the mice, while this occurred only rarely in C57BL; none of these mice carried the InuIdX marker.

The differences in inulin immune responses in inbred and Ig-congenic strains suggest a complex genetic control of expression of anti-inulin V-region genes.

Localization of Individual (IdI) and Cross-Specific Idiotypes (IdX) of Inulin-Binding Myeloma Proteins (InuBMP) to Specific Ig Chains. R. Lieberman, M. Vrana, W. Humphrey, Jr., C.C. Chien, and M. Potter. National Cancer Institute, Bethesda, Maryland 20014.

To understand the structural basis of idiotypy, it is important to correlate serological (antigenic) with structural (sequences) data among idiotypes of functionally related myeloma proteins. Accordingly, the topology of individual idiotypes (IdI) and cross-specific idiotypes (IdX) was studied in a group of BALB/c inulin-binding myeloma proteins (InuBMP). Allogeneic anti-idiotype antisera made specific for InuIdI and InuIdX by absorption were tested in an HA system with a homologous or crossreacting myeloma protein. Monomers of recombinant molecules consisting of light and heavy chains from different InuBMP were used as inhibitors of the InuIdX systems. For example, recombinant molecules E109L+U61H and E109H+U61L were used to inhibit the HA of anti-E109 IdI with E109 SRBC. Anti-E109 IdI was inhibited by E109L+U61H but not by E109H+U61L. Similarly, anti-U61 IdI reacted to U61 SRBC was inhibited by U61L+E109H and not be U61H+E109L. Neither E109 IdI nor U61 IdI is inhibitable by the hapten (fructan trisaccharide), or by the E109 or U61 light or heavy chains alone. Thus, E109-IdI and U61-IdI were determined by 109 V_L and U61 V_L, respectively, but required the appropriate pairing with V_H to be expressed. The finding that 109L+X24H or 109L+U10H recombinants fail to inhibit anti-109-IdI suggested the V_H had to be of InuBMP origin, since X24 and U10 myeloma proteins do not bind inulin.

Anti-IdX antiserum was made specific for Inu-IdXB, which is a cross-specific idiotypic determinant found on U61 and not E109. Recombinants containing U61L or U61H combined with E109H or E109L were used to block the HA of anti-Inu-IdXB with a cross-specific myeloma protein. The U61-Inu-IdXB was blocked only by 61L+109H.

Antisera specific for A4-IdXA, which is present on A4 and not on T957 myeloma proteins, was tested for inhibition with A4L+T957L and A4H+T957L. In contrast to Inu-IdXB, A4-IdXA specificity was determined by A4H and not A4L, but required light chains of A4 of T957 to be expressed.

Table 1 summarizes the data on the Ig chains involved in distinguishing the antigenic specificity of U61-IdI, U61-IdXB, and A4-IdXA idiotypes. These data suggest that antigenic differences present on the light chains of E109, U61, T957, and probably A4 are responsible for the serological differences in IdIs of these four proteins, although heavy chains of InuBMP are required for their configuration. The U61-Inu-IdXB antigenic specificity also appears to be a U61L specificity, while the A4-Inu-IdXA antigenic specificity is associated with the A4H. However, both U61L and A4H require pairing to the appropriate V_H and V_L, respectively, to be expressed.

The Structural Basis of an Idiotype on an Inulin-Binding Myeloma Protein. Mark Vrana, Stuart Rudikoff, and Michael Potter. National Cancer Institute, Laboratory of Cell Biology, Bethesda, Maryland 20014.

Table 1. Localization to Specific Ig Chains of Individual and Cross-Specific Idiotypes of Inulin-binding Myeloma Proteins

	IdI-61 Anti-61:61 Abs. 109	IdX B (Inulin Class Reaction) Anti-803:3082 Abs. 109	IdX A-Like Anti-109:4 Abs. 957
Myeloma Inhibitors	U61	U61, T803, W3082	A4, W3082, T803, U61, E109, A47N
Recombinant Inhibitors	61L)109H, 61L)61H	61L)109H, 61L)61H	4H) 4L, 4H) T957L
Antibody to Inulin	–	+	+
Hapten	–	+	+
	Determined by V_L Requires V_H for display	Determined by V_L Requires V_H for display	Determined by V_H Requires V_L for display
Noninhibitors	61L 109L:61H	61L 109L:61H	4H 957H:4L

We recently described a complex system of closely related cross-specific (IdX) and individual (IdI) idiotypes in a group of myeloma proteins which bind inulin. It has become apparent that a better understanding of the structural basis of idiotypy could be accomplished by correlating idiotypic differences between proteins with their differences in primary sequence. In these abstracts, Lieberman describes the results of chain recombination between four inulin-binding proteins. The IdI of EPC-109 and the IdI and hapten-inhibitable IdxB of UPC-61 were found to depend primarily on their light chains.

In order to assess the structural basis for these idiotypes, a hypothetical model of these proteins was constructed using light chain sequences from three proteins – EPC-109, UPC-61, and ABE-47N. A total of six positions in the three light chains show amino acid replacements. Four of these are in the hypervariable regions and two are in the framework. Based on the molecular model of EPC-109, all of the positions which show variability are external residues and could result in idiotypic differences between proteins. The IdxB of UPC-61 is not found in EPC-109 and ABE-47, and so must result from one or several amino acids unique to UPC-61 light chain. The most likely candidate for the IdxB would be the two hypervariable region residues at position 30 and 92, which are approximately five angstroms apart on the molecular model. Lieberman (these abstracts) has pointed out that these idiotypic differences which result from structural differences between the light chains need an anti-inulin heavy chain for expression. In addition, the occurrence of natural antibodies to inulin in different mouse strains revealed that the IdxB is not a V_H marker, but a marker for a determinant the expression of which correlates with light-chain amino acid substitutions. This result suggests that different mouse strains which can express the same V regions may have serological markers which are linked to allotype, but are, in fact, determined by light chains. It is unclear whether other idiotypes linked to allotype are indeed V_H markers or depend on a particular V_L for expression.

Binding Studies on Antifructofuranan Mouse Myeloma Immunoglobulins A47N, U61, and E109. D.G. Streefkerk and C.P.J. Glaudemans. National Institutes of Health, Bethesda, Maryland 20014.

Four murine myeloma immunoglobulins – A4, A47N, U61, and E109 – have been studied for their binding affinities with inulin and a series of oligosaccharides derived from inulin. The results indicate that the combining site of these immunoglobulins show highest complementarity for a trifructofuranosyl sequence (A4 and A47N) and a tetrafructofuranosyl sequence (U61 and E109). The size of the combining area of the immunoglobulin E109 derived from the antigenic determinant (approximately 15 × 14 × 10 Å) agrees well with the size observed on a hypothetical space model

of the Fv portion of E109 (M. Potter, E. Haber, and R.M. Krause, Eds.): *In*: *Antibodies in Human Diagnosis and Therapy*. Raven Press, New York, 1977), where the virtual absence of H3 in E109 opens up a large combining area capable of accommodating the bulky 2→6-linked fructofuranosyl oligomer. For this reason, it was proposed that the extended, ribbon-like, 2→1-linked fructofuranan could bind to the anti-(2→6 fructans), but not vice versa.

Binding Site-Dependent Anti-Idiotypes Raised Against Proteins 460 and 315 as V-Region Probes. R.W. Rosenstein, J.B. Zeldis, and F.F. Richards. Yale University Medical School, New Haven, Connecticut 06510.

Hapten-inhibitable, anti-idiotypic reagents were raised in rabbits against proteins 460 and 315, two IgA myeloma immunoglobulins which bind the haptens dinitrophenyl and menadione. Anti-idiotypes were purified from 460 and 315-sepharose affinity columns by hapten elution. Early bleeds (one month) of the immunized rabbits yielded a population of anti-idiotypes consisting of both "private" 460 or 315 reagents and also reagents which shared specificity for 460 and 315. Later bleeds (three to five months) yielded almost exclusively "private" idiotypes.

When BALB/c mice were immunized with dnp-BGG, 460 idiotype-positive immunoglobulins could be detected. They were predominantly of IgG_1 and IgA types, although smaller amounts of IgM, IgG_{2a}, and IgG_{2b} could also be detected. The IgA fraction had 460 "private" and shared idiotypes, while IgG_1 had only the 460 "private" idiotypic marker. Protein 315 idiotype could not be detected in immunized BALB/c serum.

The 460 idiotypes could also be detected in other mouse strains sharing the Igl^{a+} allotype (BALB/c, SJA, B·C-9, C58/J). We were unable to detect 315 idiotypes in any of the strains tested, including strains related to the congenic mouse in which protein 315 first arose (C57BL/6 and BALB/c).

Mapping of V_H-Region Genes for Antinuclease Antibodies. David S. Pisetsky and David H. Sachs. Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

Antibodies to *Staphylococcal* nuclease have been fractionated into two populations on the basis of ability to bind to the CNBr cleavage product of nuclease comprising the C-terminal portion of the molecule from the 99th to the 149th amino acid. The two antibody populations, antinuclease (1–99) and antinuclease (99–149), have been prepared from strains A/J, SJL, BALB/c, CB.20, and BAB.14, and analyzed using anti-idiotypic antisera raised against whole antinuclease antibodies from strains A/J, SJL, BALB/c, and B10.A(2R). For all strains examined, antinuclease (1–99) antibodies had the same pattern of reactivity with the anti-idiotypic antisera as did unfractionated antibodies. A different pattern was found for antinuclease (99–149) antibodies in some instances. These results indicate that the two antibody subpopulations bear distinct idiotypic markers. From analysis of the distribution of these markers in strains BALB/c, CB.20, and BAB.14, maps of genes determining these idiotypic markers have been constructed. These maps support the idea that the recombination event that occurred during the development of the BAB.14 strain separated V_H-region genes.

Idiotypes on Galactan-Binding Myeloma Proteins and Antigalactan Antibodies in Mice. E.B. Mushinski and M. Potter. Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland 20014.

Idiotypic antibodies were induced in rabbits to β1,6D-galactan-binding myeloma proteins (GALBMP). Selected antisera identified cross-specific idiotypes (IdX) shared on two or more of the eight available GALBMP. The IdX of GALBMP were not found on 118 other myeloma proteins screened. Galactan-binding antibodies were induced in four mouse strains (BALB/c, C57BL/Ka, CB-20, and BC-8) by i.v. injection of gum ghatti, a polysaccharide containing β1,6-linked D-galactoses. Antigum ghatti antibodies shared most of the specific IdX determinants of GALBMP. These antigum ghatti antibodies were tested for immunoglobulin class specificities and found to be made up of IgA, IgG, and IgM classes.

Individual idiotypes (IdI) of seven of the GALBMP were prepared in strain AL/N mice. One of these idiotypes, IdI-XRPC24, was found in antigum ghatti antibody by inhibition of hemagglutination. All GALBMP thus far have the same V_κ and V_H isotype composition. The failure to find all of the IdI determinants in antigum ghatti antibody suggests that the generator of the IdI specificities is not controlled by germline genes and may be caused by somatic mutations.

Restriction in the Immune Response to Flagellar Proteins in Inbred Mice. A.M. Smith, J. Slack, and M. Potter. East Carolina University School of Medicine, Greenville, North Carolina 27834, and Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland 20014.

Anti-idiotypic sera prepared in both AL/N mice and rabbits identify specificities common to the IgA MOPC467 myeloma protein and day-seven affinity-absorbed antibodies to *Salmonella milwaukee* polymerized flagellin (S.mil-POL) raised in BALB/c and C57BL/Ka mice. Isoelectric focusing (IEF) of reduced and alkylated BALB/c anti-S.mil-POL gave a banding pattern of L and H chains identical to MOPC467. C57BL/Ka anti-S.mil-POL had a similar L-chain pattern but a different H-chain pattern. Both BALB/c and C57BL/Ka contain predominantly IgA. The IEF patterns in the two strains are consistent with a monoclonal response at day seven.

Identification and Variable Penetrance of Multiple, Linked Idiotypes Among Murine Antidextran Antibodies. Daniel Hansburg, David E. Briles, Joseph M. Davie, and Michael Potter. Department of Microbiology and Immunology, Washington University Medical School, St. Louis, Missouri 63130, and Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland 20014.

Idiotypic assays specific for the B1355 dextran-binding myeloma proteins (DBMP), M104, J558, and U102, and an additional assay specific for determinants common to all DBMPs were constructed. Using these assays, it was shown that there are at least five species of antidextran antibody – three possessing the myeloma-specific idiotypes (IdIs), one possessing the common idiotype (IdX) but lacking any IdI, and one lacking any IdI and quantitatively deficient in IdX but possessing the lambda light chain. Six murine strains – BALB/c, BAB.14, C57L, C58/J, 129/J, and BRVR – were examined for these idiotypes. In all strains, each of the five species of antibody was expressed. The prevalence of the species varied widely; the IdX+/IdI antibodies were more common than the IdI (M104), which in turn was more common than the IdI (J558) and IdI (U102). An exception to this distribution was noted among BRVR, in which the expression of the IdX+ and IdI (M104) was reduced. The conclusion of multiple, distinct molecular species of antibody is corroborated by analytic isoelectric focusing with radiolabeled antigen visualization. In strains BALB/c, BAB.14, and C58/J a clear association of focusing pattern and the ratio of IdX+/IdX was noted among both 19S and 7S antibodies. However, other idiotypic differences have not been correlated with changes in the focus pattern. Thus, the same spectrotype isolated from different individuals was shown to have a markedly variable amount of IdI (M104). Since large amounts of antidextran antibody are available by the novel immunization protocol used, CB.20 and C57BL/6 were examined. In the more than ten individuals from each strain examined, the IdX idiotype was not detected. (Thanks are due to Dr. L.A. Herzenberg for his gift of BAB.14 mice.)

Genetics of the Immune Response to the Streptococcal Group A Polysaccharide in Mice. D.G. Braun. Basel Institute for Immunology, Basel, Switzerland.

We thought previously that antibody levels to A-CHO were controlled by a single autosomal gene, *Ir-A-CHO*, with three alleles. The evidence available now suggests that at least three genes are involved, *i.e.*, control is multigenic. One of these genes is linked to allotype and controls the expression of Ig subclasses in A-CHO-specific antibodies. For example, high responder BALB/c mice respond primarily to A-CHO with IgG_{2a} antibodies, whereas low responder C57BL/6 mice respond with IgG_{2b} and IgG_1 antibodies. BALB/c-congenic CB-20 mice, which carry genes for both variable and constant regions from C57BL/Ka, behave as low responders with IgG_{2b} and IgG_1 antibodies being expressed.

Extensive analyses were performed with hyperimmune sera of eight Bailey RI lines derived from breeding stocks of (BALB/c × C57BL/F_2 mice: C × BD, C × BE, C × BG, C × BH, C × BI, C × BJ, C × BJaw, and C × BK. As previously shown, antibody levels are independent of the *H-2* haplotype. These RI lines can be classified into three responder groups according to their levels of anti-A-CHO antibody: high (C × BJ, C × BK), heterogeneous high-low (C × BD, C × BG, C × BH, C × BJaw), and low (C × BE, C × BI). Within the high-low responder lines, low and high responder mice may express a given clonotype at levels which are greater than a hundred fold. Like idiotypes, clonotype patterns are associated with Ig allotypes. Since A-CHO-binding clonotypes as well as the S117 idiotype were associated with Ig allotypes, recurrent clonotype patterns of both BALB/c (high responder) and C57BL/6 (low responder) origin could be identified, and the position of the S117 idiotype was determined by its pI. Seven clonotypes of BALB/c origin (including the S117 idiotype) were identified in RI lines C × BG, C × BJ, and C × BJaw; ten clonotypes of C57BL/6

mice were identified in strains C × BD, C × BE, C × BI, C × BH, and C × BK. This analysis documents that mice show heritable differences for at least 17 clonotypes which are specific for the N-acetylglucosamine, the determinant of the group A polysaccharide. Furthermore, this analysis makes it unlikely that the level of responsiveness is controlled by either the V_H or the V_L domain. Much of the data available in the A-CHO system point to a critical function of the C_H region (at the subgroup level of expression) in controlling high and low responses. The level of clonotype expression is probably regulated by epigenetic factors, *e.g.*, the immunological history of an animal. It is, however, conceivable that clonotype expression may depend on subclass association.

(This work was made possible by Dr. M. Potter's (NCI/NIH) gift of congenic mouse strains. Dr. K. Eichmann, German Cancer Research Center, Heidelberg, determined the S117 idiotype.)

Screening of Mouse Plasmacytomas for Binding Capacity of Haptens. O. Mäkelä and M. Potter. Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland 20014.

We screened 239 ascites fluids of plasmacytoma-carrying mice for their capacity to inactivate haptenated bacteriophages (an indication of hapten-binding capacity).

The bacteriophages used were coupled with one of 13 compounds, and all myelomas were tested with all 13 phages. The compounds fell into four unrelated families, but members of one family can be very distant. For instance, haptens DIP and NP have no detectable immunological crossreactivity. They are relatives because both can be shown to be relatives of NIP. The compounds were:

NP-cap	DNP	BOC-ABA-Tyr	Penicillin
NBrP-cap		ABA-NP	Oxacillin
NIP-cap		ABA-MIP	
NNP		PAB-HOP	
DIP		ABS-HOP	

Eight plasmacytomas screened were hapten binders. Their inactivation titers with selected phages are given below.

	NIP-cap-T4	DNP-cap-T4	ABA-MIP-T4
ABPC-24 IgA λ	4,000,000	<10,000	<300
EPC-26 IgA	6,000,000	<10,000	<300
ALPC-46 IgA	10,000,000	<10,000	<300
CBPC-14	<10,000	<10,000	60,000
CBPC-38 IgA	<10,000	60,000,000	<300
CBPC-106 IgA	<10,000	3,000,000	<300
CBPC-108 IgH	<10,000	70,000	<300
CBPC-111	<10,000	50,000	<300
(MOPC-315, reference)	<10,000	80,000,000	<300

Rabbit Ig Genetics: An Overview. Rose G. Mage, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014.

In addition to the intrinsic interest in the subject of rabbit Ig genetics, results in this system are relevant to many of the problems raised by current data on mouse Ig genetics. As with some anti-idiotype reagents, anti-*a*-allotype (V_H) reagents recognize a group of structurally related V_H regions. The usual anti-*a*-allotype reagents probably recognize more than one antigenic determinant per V_H (Fab), and their specificity and crossreactivity is influenced by the particular donor-recipient combination used to elicit them. Individual antibodies of limited heterogeneity may be deficient in their ability to compete with the binding of labeled pooled IgG to insolubilized anti-*a* allotype antibodies or to inhibit the binding of labeled anti-*a* allotype antibodies to insolubilized IgG. The deficiency of the inhibitors compared to normal IgG may be accounted for by total absence of certain determinants or by the presence of determinants to which anti-*a* allotype antibodies bind with lower average association constants. Residue positions at which structural correlates of *a* allotypes occur are distributed through much of the V_H framework. In the folded molecule,

some of these positions are brought into close proximity. The haplotypes of rabbit populations and of two of three reported recombinants between V_H and C_γ allotypes provide no evidence that recombinations have occurred between V_H and C_μ genes. It will, therefore, be of considerable interest to haplotype fully the third recombinant family and to determine the status of μ and γ markers in mice in which apparent recombinations within the heavy-chain linkage group occurred.

Sequence of the Variable Region of a Homogeneous Rabbit Antibody Light Chain. Alberto Chersi, Ettore Appella, Salvatore Carta, and Rose Mage. National Institute of Allergy and Infectious Diseases, and National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

The variable region of the homogeneous light chain of a rabbit antibody has been sequenced by automatic Edman degradation. The rabbit AH80-5 ($a^3a^3b^4b^4$) was immunized with Type III pneumococcal vaccine. The highly restricted antibody was purified by DEAE chromatography, and the light chain was obtained by gel filtration after mild reduction and alkylation of the IgG.

Trypsin digestion of the carboxymethylated, fully succinylated light chain yielded four main fragments, the biggest corresponding to the whole constant region, the three others covering the whole variable region.

The three fragments of the variable region were almost completely sequenced by automatic Edman degradation with a Beckman sequencer. The AH80-5 light chain shows striking similarities with light chains of rabbit antistreptococcal group A-variant antibodies which have amino terminal Ile at homology position 2. (D.G. Braun *et al.*: *Eur. J. Immunol.* *6*:570, 1976). There are at least four differences in sequence up to position 88, as indicated below:

Residue number	26	34	46	50
AH80-5 L	Ala	Ser	Gly	Tyr
Anti-A-Var L	Ser	Ala	Leu	Lys.

There is complete identity in sequence of positions 27 through 33 of the first hypervariable region. The third hypervariable region of this light chain, however, is quite different from any of the four reported for antistreptococcal A-variant antibodies.

From the amino acid composition of the tryptic fragment covering positions 33 through 61, which was sequenced as far as Val 58, there is an indication that this light chain may have two or three additional residues (two Glx and possibly an Asx) inserted after this amino acid. This is near where light chains of many species have the second hypervariable region. Rabbit kappa chains, however, have been remarkably invariant in sequence in this region.

Selective Expression of Antibodies Structurally Related to, But Not Identical with, the Phosphorylcholine-Binding Myeloma Protein, MCPC 603. Katie Williams and Latham Claflin. Department of Microbiology, The University of Michigan, Ann Arbor, Michigan 48109.

The IgM response to phosphorylcholine (PC) following immunization with *S. pneumoniae* (R36A) exhibits two major characteristics. First, clones dominating the response to PC comprise a limited set, *i.e.*, anti-PC antibodies from genetically diverse mice have idiotypic and structural features equivalent to those of the BALB/c PC-binding myeloma proteins T15 and M511, but not to M167 or W3207. Second, the response to PC is conserved; a set of remarkably similar clones is expressed in each strain. The exception has been BALB/c, which expresses anti-PC antibody dominated by a single clonotype, T15. Recently, we have observed the expression of non-T15, but M603-like clones of the IgG, as well as the IgM, class in mice immunized with *Proteus morganii* isolated from mice by M. Potter. Cell-free extracts of this organism are bound by M603 500-times greater than by any of the other PC-binding myeloma proteins. Immunization with *P. morganii* stimulates an isoelectric focusing or spectrotype pattern that is complex but of limited heterogeneity. Repetitive patterns are seen, but individual mice may differ considerably. The T15 and M511 idiotypes, as detected with heterologous antisite-specific antisera, and the corresponding T15 and M511 light (L) chains appear after immunization. Whereas T15 is the dominant clonotype following immunization with R36A or PC-KLH, it comprises only between 0.7 and 25 percent of the PC-specific antibody in *P. morganii*-immune mice. Thus, BALB/c can readily express clonotypes other than T15 which in some may dominate the response. A second feature of the anti-PC response to *P. morganii* is that the vast majority of antibodies bear an L chain which cofocuses

with M603 L chain. However, quantitative idiotypic analysis with binding site- and nonbinding site-specific antisera of 30 different mice fails to detect the M603 idiotype. This implies that some anti-PC antibodies contain an immunoglobulin with an L chain similar to that of M603, but with a different H chain. The virtual absence of the M603 idiotype suggests either that the M603 myeloma idiotype is not readily expressed even under what seem to be optimal conditions, or the intriguing possibility that M603 may actually represent a somatic permutation of existing germline information.

Antigen-Binding Variants of S107. Wendy D. Cook and Matthew D. Scharff. Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461.

S107, a tissue culture-adapted line of mouse myeloma cells (obtained from the Salk Institute) was cloned and the subclones were examined for antigen-binding variants. Specifically, cells were cloned in soft agar and overlaid with phosphocholine attached to KLH (PC-KLH). Parental clones secreted their immunoglobulin into the surrounding agar, where it reacted with the PC-KLH to form a precipitate. In different experiments, between 0.1 and 1 percent of the clones were not surrounded by precipitate. In one experiment, 0.9 percent of the clones did not "stain" with antigen, while 0.3 percent were negative when the clones were overlaid with anti-IgA. This suggested the presence of variants which secreted IgA but failed to bind antigen. Three such variant clones were recovered and grown to mass culture. Only one variant has been characterized in detail: 1) the protein produced by the variant still binds PC but a tenfold excess of the variant protein is required to compete with labeled parental protein for PC binding; 2) the variant synthesizes and secretes approximately the same amount of IgA as the parent; 3) the size of the variant and parental H and L chains are identical; 4) polymers are secreted by both variant and parent; and 5) the variant L chains are identical to those of the parent by peptide maps, but the heavy chains differ by a few peptides. When this variant was recloned, it generated antigen-binding phenotypic revertants at a frequency of 0.2 to 2 percent. We conclude that the S107 mouse myeloma cell line spontaneously generates variants which have changes in antigen binding. The molecular basis for these changes will require detailed sequence comparison of the variant and parental immunoglobulin. (Supported by grants from the NIH, NSF, and the Young Men's Philanthropic League.)

Parameters of T15 Idiotype Expression in Allotypically Distinct Strains of Mice. Michael P. Cancro, Nolan H. Sigal, and Norman R. Klinman. Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104.

The primary antiphosphorylcholine (PC) response in BALB/c, C57BL/6, and congenic and recombinant inbred strains of these parental types was examined in the splenic focus system. The frequencies of PC-specific precursors was shown to vary among these strains from two to 20 precursors per 10^6 splenic B cells (Table 2). The distribution of these frequencies suggests that elements closely linked to or within the major histocompatibility complex may play a role in the determination of this parameter, although additional experiments are necessary to assess this possibility adequately. More interestingly, all strains tested, regardless of immunoglobulin allotype, expressed monoclonal antibodies indistinguishable from the T15 clonotype. Further, the frequency of this clonotype in a given strain did not appear related to allotype, since both high and low T15 frequencies may be found among strains of either the a^1 or a^2 allotype. The examination of normal serum for T15 levels, in agreement with the work of other laboratories (Lieberman, *et al.*: *J. Exp. Med. 139*:983, 1974) reveals that only mice of the BALB/c allotype (a^1) expressed the T15 idiotype (Table 2) in detectable quantities.

The results in Table 2 suggest that the allotype of an individual, although closely related to serum levels of an idiotype, is unrelated to the proportion of the precursor population which expresses that idiotype, and that the serum expression of a given idiotype may reflect regulatory processes which act during or before antigenic stimulation rather than the actual clonotype representation in the repertoire. These findings indicate that distinctions must be made between the expression of idiotypic determinants within precursor B-cell populations and elements which regulate the subsequent appearance of those idiotypes in serum antibody.

Presence of the T15 Clonotype in Mouse Strains Differing in Ig C_H Allotype. P.J. Gearhart and J.J. Cebra. Department of Biology, The Johns Hopkins University, Baltimore Maryland 21218.

Table 2. Summary of T15 PC-Specific Precursors and Normal Serum Antibody T15 Levels in BALB/c, C57BL/6, Recombinant Inbred, and Congenic Strains

Strain	*H-2*	Allotype	PC-Specific Precursors per 10^6 Splenic B Cells	T15-Positive Precursors per 10^6 Splenic B Cells	Normal Serum T15 (mcg/ml)
BALB/c	*d*	a^1	16	10	17
CB20	*d*	a^2	23	8	3
B10.D2	*d*	a^2	11	1	3
CxBD	*d*	a^2	15	2	3
CxBH	*d*	a^2	16	7	3
CxBE	*b*	a^2	2	0.6	3
CxBG	*b*	a^1	2	0.7	13
CxBI	*b*	a^2	3	0.6	3
CxBJ	*b*	a^1	3	2	25
CxBK	*b*	a^2	4	0.6	3
C57BL/6	*b*	a^2	2	1	3
(BALB/c × C57BL/6)F_1	*d/b*	a^1/a^2	16	5	N.D.

The immune response to phosphorylcholine (PC) in BALB/c mice is dominated by a single clonotype which is identical to that of cells producing the TEPC 15 (T15) myeloma protein. The antibody products of this clonotype are identical to T15 protein by the criteria of complete idiotypic crossreactivity, ligand-binding specificity, and isoelectric focusing. Some 70 percent of splenic B cells responsive to PC belong to this clonotype, and the serum response reflects this predominance in that the majority of antibody produced to the PC determinant has the T15 idiotype. Among the BALB/c non-T15 repertoire, we have identified several clonotypes by partial idiotypic crossreactivity and by affinity for hapten. One of these is a monoclonal antibody which reacts with a hapten-inhibitable heterologous anti-T15, but has no reaction with murine nonhapten-inhibitable anti-T15 (*J. Exp. Med. 145*:876, 1977). In a study of nonimmune and immune serum antibodies, Lieberman and coworkers have reported an Ig-1a allotype-linked strain distribution of the T15 marker as identified by murine anti-T15 antibody. Claflin and Davie have demonstrated a binding site-associated idiotypic marker that is shared among anti-PC antibodies from all mouse strains.

However, the analysis of idiotypic relationships among serum antibodies may be ambiguous in that detection of low levels of an idiotype may indicate weak crossreactions among a heterogeneous population of antibodies. Secondly, serum antibody represents the expression of an antigen-selected population of B cells. Thus, absent or low levels of an idiotype may not be related to the actual B-cell repertoire. The in vitro analysis of individual B cells by the splenic focus assay of Klinman has proved to be a powerful tool for identifying homogeneous antibody as belonging to a particular clonotype, determining the frequency of clonotypes, and the detecting minor populations of B cells which may not be stimulated in vivo. In this regard, the individual analysis of B cells is the most rigorous method for determining whether a particular V gene may be expressed. We have previously shown using both hapten- and nonhapten-inhibitable anti-idiotypes that 30 percent of PC-specific B cells from the AKR (Ig-1d) strain produce antibody which is idiotypically indistinguishable from the BALB/c T15 clonotype (*J. Exp. Med. 145*:876, 1977). This report extends the finding in that the T15 clonotype is represented among precursors from congenic mice with the Ig-1b and Ig-1d allotypes, as well as from C57BL/10 mice, indicating a remarkable sharing of T15 variable regions among allotypically distinct strains.

Anti-T15 was raised in A/He mice and absorbed by MOPC 460 to remove antiallotype activity. Using a solid-phase inhibition radioimmunoassay, the anti-T15 from a single mouse used in this study had no specificity for MOPC 167 or McPC 603 but had 50 percent crossreactivity on a weight basis with MOPC 511. Monoclonal antibodies were obtained from in vitro splenic fragment cultures of donor spleen cells that had been transferred into carrier-primed syngeneic or histocompatible congenic recipients. Total antibody was determined in both a radioimmunoassay which detected

antibody bound to PC conjugated to an immunoabsorbant by means of ^{125}I-labeled anti-Fab, and the anti-idiotype assay. Both assays were standardized with T15 protein. The criterion for idiotypic identity was a ratio of nanograms of antibody detected by anti-Fab versus nanograms of antibody detected by anti-T15 of less than 1.4. Thus, crossreactive antibody, such as MOPC 511, would not be termed idiotype-positive by these criteria; only antibody that reacted as efficiently as T15 in the antiidiotype inhibition assay was identified as belonging to the T15 clonotype.

The data from B cells of the C57BL/10, B10.D2, CB20, and CAL.20 strains indicate that the frequency of the T15 clonotype ranges from two to 20 precursors per 10^6 B cells, representing 25 to 70 percent of the anti-PC-specific repertoire. The T15-positive monoclonal antibodies from these mice showed the same narrow range of hapten inhibition of binding to antigen as BALB/c T15-positive antibody; a wide range of hapten inhibition of binding was found for non-T15 antibodies. Using a radioimmunoassay to detect the Ig-2a allotype, we confirmed the lack of association between the T15 idiotype marker and the heavy-chain IgA allotype marker. All of the BALB/c clones that produced IgA antibody had the Ig-2a allotypic determinant, whereas none of the T15-positive clones from C57BL/10, B10.D2, CB20, or CAL.20 that produced IgA had the Ig-2a allotype.

These results of the analysis of idiotypic antibodies produced by antigen-stimulated B cells from nonimmune populations of various mouse strains suggest that the repertoire of available clonotypes may be larger than those specificities that are readily detected in selected populations of serum antibody. The T15 idiotype presumably represents the expression of a germline V gene, since every mouse expressed it. Thus, the number of germline specificities may be larger than deduced from analysis of serum immunoglobulins. The results also suggest that we should use caution in interpreting the presence or absence of an idiotypic marker in normal or immune sera as an indication of the existence and chromosome location of a V gene, particularly in the construction of V-region maps. (Supported by NIAID grant AI-09652, NSF grant GB-38798, the Dupont Corporation, and Helen H. Whitney Foundation.)

Search for the Chromosomal Location of the Immunoglobulin Heavy-Chain Complex of the Mouse. B.A. Taylor and E.M. Eicher. The Jackson Laboratory, Bar Harbor, Maine 04609.

All efforts to map the heavy-chain allotype locus (*Ig-1*) in the mouse have been unsuccessful. Linkage between *Ig-1* and the serum prealbumin locus (*Pre-1*) has been described (B.A. Taylor, *et al.*: *Nature 256*:644–646). The distance between *Ig-1* and *Pre-1* is approximately ten map units. We have used the *Pre-1* marker in various linkage tests in an effort to assign this linkage group to a chromosome. The chromosomes and markers that we have tested are as follows: Chr 1 (*Id-1, ln, Dip-1*); Chr 2 (*Sd*); Chr 3 (*Car-2, my*); Chr 4 (*b, Gpd-1*); Chr 5 (*Hm*); Chr 8 (*Gr-1, Es-1, e*); Chr 10 (*Sl*); Chr 11 (*Re*); Chr 12 (*Amy-1, cdm, Va*); Chr 13 (*Xt*); Chr 15 (*uw, Gpt-1, bt, Ca*); Chr 16 and 17 (Rb(16.17)7Bnr); Chr 18 (*Tw*); and Chr 19 (*Got-1*). We have reviewed all of the available negative linkage data to determine the remaining chromosomal regions in which *Ig-1* and *Pre-1* might reside. They are the distal parts of chromosomes 3, 13, and 16, the proximal end of chromosome 4, and chromosome 18. We plan to use translocation stocks to test for linkage to these regions where useful genes are not available. (Supported in part by contract N01 CP 33255 within the Virus Cancer Program of the National Cancer Institute and NIH grant CM 20919.)

A Possible Allotype-Linked Histocompatibility Gene. Roy Riblet and Cecil Congleton. The Institute for Cancer Research, Philadelphia, Pennsylvania 19111.

Reciprocal skin grafts between allotype-congenic mouse strains revealed a weak incompatibility. C.B-17 and C.B-26 mice rejected BALB/c skin with a median survival time of approximately 60 days. In the reciprocal exchange, BALB/c mice rejected C.B skin with a medium survival of over 200 days. Skin survival can be shortened by preimmunization with spleen cells.

The existence of this difference in congenic strains is strong evidence of allotype linkage, but the possibility that this difference is caused by an unlinked mutation in one of the strains or the fortuitous inheritance of an unlinked difference between the two progenitor strains is being tested.

Analysis of congenic strains, including several which are V-C crossovers suggests that this histocompatibility locus is located between C_H and Pre, giving the following map for the heavy chain region: V_H-C_H-H(Ig)-Pre.

Unusual Genetic Features of the Heavy-Chain Complex Locus in the Mouse. Roy Riblet and David Boyer. The Institute for Cancer Research, Philadelphia, Pennsylvania 19111.

Mice from several backcrosses have been screened to identify genetic crossovers between V_HDEX and C_H. V_HDEX is the heavy-chain variable region gene coding for antidextran antibodies with idiotypes crossreactive with the antidextran myeloma proteins J558 and MOPC-104E. C_H represents the closely linked allotype cluster Ig-1 through Ig-6, coding for the heavy-chain constant regions of mouse antibodies. A total of 14 crossovers have been found in 3544 segregating mice, giving a map distance of 0.4 percent between V_HDEX and the C_H cluster. The recombination rate was similar in all crosses. Although some of these crossovers initially appeared to be unequal events, further analysis disproved this, and there is as yet no evidence for such events in this chromosomal region. Hansburg and coworkers (*J. Immunol. 117*:569, 1976) have suggested that the V_HDEX gene is, in fact, several genes coding for at least three cross reactive but distinct idiotypes in the antidextran response. The existence of multiple genes would be proved by a crossover which separates them, but in this group of crossovers the mixture of idiotypes appeared to be gained or lost as a unit. This suggests that if there are several genes comprising V_HDEX, they must be clustered closely together.

These crossovers revealed several unusual genetic features of the array of V_H and C_H genes which form the heavy-chain complex locus. First, the two types of recombinant were not found in the expected equal numbers. In a cross of V_HDEX^+, Ig^b by V_HDEX^-, Ig^d, five V_HDEX^-, Ig^b recombinants were found, but no V_HDEX^+, Ig^d crossovers. Similarly, another cross yielded five recombinants of one type, but only one of the reciprocal kind. Second, a high frequency of multiple crossingover was found. Of 12 V-C crossovers which were also typed for prealbumin, five had also recombined in the C-Pre segment, which is ten map units long. This frequency of 42 percent, rather than the expected ten percent, and the biased yields of crossover types suggest that the heavy-chain complex locus contains unusual genetic structures which promote repeated exchanges but restrict the directions in which they can occur. These structures presumably are the signals or substrate sites for the enzymes of the excision/insertion or recombination processes of V-C translocation and sequence diversification.

II. Genetic Control of Light Chains

Genetic Control of the Antibody Response of Inbred Mice to 4-Hydroxy-3-Nitrophenylacetyl (NP) Group. T. Imanishi-Kari, R.S. Jack, M. Reth, and K. Rajewsky. Institute for Genetics, University of Cologne, Weyertal 121, D-5000 Köln 41, Federal Republic of Germany.

Strain C57BL/6 mice produce highly restricted primary response to the 4-hydroxy-3-nitrophenylacetyl (NP) group (A.J. McMichael, J.M. Phillips, A.R. Williams, T. Imanishi, and O. Mäkelä: *Immunogenetics 2*:161, 1975). This response is composed of molecules having μ and γ_1 heavy chains and light chains of the lambda type. The anti-NP antibodies carry a characteristically fine specificity marker, *i.e.*, bind the crossreacting hapten NIP (4-hydroxy-5-iodo-3-nitrophenylacetyl) with a higher affinity than does the homologous hapten NP (heteroclitic) (T. Imanishi and O. Mäkelä: *Eur. J. Immunol. 3*:323, 1973), and can also be idiotypically defined. A guinea pig anti-idiotypic antiserum was raised against the purified C57BL/6 primary anti-NP immunoglobulin. This anti-idiotype was shown to detect markers present on the primary anti-NP immunoglobulin only in those strains which express the $Ig\text{-}1^b$ allotype. In these strains, more than 90 percent of the anti-NP antibodies in the primary response were bound by the anti-idiotypic antiserum.

The idiotypic determinants of primary anti-NP antibodies from C57BL/6 mice were shown to be controlled by a gene (or genes) linked to the heavy-chain allotype locus in breeding experiments using $(C57BL/6 \times CBA)F_1 \times CBA$ backcross, $(C57BL/6 \times CBA)F_2$, the congenic CB-20 inbred strain, and the Bailey recombinant inbred strains. With the exception of strain A, which express a weakly crossreactive idiotype, idiotype-positive responses were not found in any strain other than those carrying the $Ig\text{-}1^b$ allotype. Idiotype-positive responses were always associated with the predominant expression of lambda chains in the NP-binding antibody molecules. The anti-NP response of idiotype negative strains consisted mainly of antibody molecules carrying kappa light chains.

Within the group of $Ig\text{-}1^b$ strains, only strain SJL mice failed to produce an idiotype-positive response. SJL animals also constitute the only $Ig\text{-}1^b$ strain so far tested which fails to produce

heteroclitic anti-NP antibodies, and in addition is known to produce only five percent of the normal amount of light chains of the lambda type (W. Geckeler and M. Cohn: *Immunogenetics* *4*:429, 1977, and these abstracts). One possible interpretation of these data is that all Ig-1^b strains including SJL carry a germline V_H gene, the product of which represents part of an idiotype-positive, heteroclitic, NP-specific binding site if combined with the variable domain of a lambda light chain. Combination of the same V_H domain with a V_κ domain usually results in a molecule either with little or no affinity for NP or, alternatively, an anti-NP molecule which is neither heteroclitic nor idiotype-positive. These possibilities were investigated by crossing strain SJL with strain BALB/c. BALB/c mice produce nonheteroclitic and idiotype-negative anti-NP antibodies, but are known to produce normal amounts of lambda-chain type (W. Geckeler and M. Cohn: *Immunogenetics* *4*:429, 1977, and these abstracts).

The (SJL × BALB/c)F_1 mice produced heteroclitic and idiotype-positive anti-NP antibodies.

In the breeding experiments, the idiotypic marker of C57BL/6 primary anti-NP antibodies is genetically linked to the *Ig-1* complex, and so the serological determinants detected by the anti-idiotype appear to be present on the heavy chain. However, if both strains CBA and C57BL/6 possess the appropriate light chains, the marker genetically defined in these experiments is still a V_H gene marker, though the serological determinants detected by the anti-idiotype may be exclusively on V_L, exclusively on V_H, or on both.

The restricted antibody pattern in the primary anti-NP response contrasts strikingly with the potential of hyperimmune C57BL/6 mice to express a large variety of anti-NP antibodies of various classes, carrying kappa or lambda light chains and carrying a large range of affinities for the hapten.

Nine continuous hybrid cell lines were produced by the Kohler-Milstein technique. Each produced one particular species of such anti-NP antibodies from C57BL/6 hyperimmunized mice (M. Reth, T. Imanishi-Kari, R.S. Jack, M. Cramer, U. Krawinkel, G.J. Hämmerling, and K. Rajewsky: *In* E. Sercarz, L. Herzenberg, and C.F. Fox (eds): *The Immune System: Genes and the Cells in Which They Function,* ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. VIII. Academic Press, New York, in press, 1977).

So far, our results indicate that all the hybridoma antibodies are idiotypically distinct from the primary anti-NP antibodies. This is also true for those antibodies that express a fine specificity similar to that of the primary response antibody. However, a striking correlation of heteroclicity with the presence of lambda light chains in the antibody molecule was found.

It is on the basis of these results that we consider it more likely that our anti-idiotypic antisera (which do not crossreact with the other lambda-bearing molecules) recognize determinants on the heavy chain of the primary anti-NP antibody or that the idiotypic determinants are characteristic for a certain combination of heavy and light chains.

The Effect of V_L on the Inheritance of an Idiotype Associated with Antibodies to p-Azophenylarsonate. A. Nisonoff, J.A. Laskin, A. Grey, N.R. Klinman, and P.D. Gottlieb. Rosensteil Research Center, Brandeis University, Waltham, Massachusetts 02154, University of Pennsylvania, Philadelphia, Pennsylvania 19104, and Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

In a number of investigations, the gene or genes controlling idiotypes have been found to be linked to a locus controlling the constant regions of the heavy chains of mouse immunoglobulins. Since the breeding studies were carried out without taking cognizance of the light chains, these results have appeared somewhat paradoxical, since many idiotypes require the participation of both heavy and light chains for their full expression. One possibility is that either of the pair of strains used in each study was capable of providing the light chains necessary for expression of the idiotype under investigation. Our recent results substantiate this hypothesis. The idiotype studied is that associated with antibodies to the *p*-azophenylarsonate group (anti-Ar antibodies) produced by all mice of the A/J strain. When immunized with keyhole limpet hemocyanin conjugated to the Ar hapten, 20 to 70 percent of the anti-Ar antibodies generally carry this idiotype. Breeding studies were done by mating A/J mice with mice of the C57BL, BALB/c, CBA, and PL strains. Mice of the F_1 generation were backcrossed to the non-A/J parental strain. The offspring of this backcross were tested for their capacity to produce the idiotype characteristic of A/J anti-Ar antibodies and, by the method of Bosma, for the presence of the A/J heavy-chain allotype. As expected, roughly 50 percent of the offspring carried the allotype. When BALB/c, C57BL, or CBA mice were used as the second strain, the results were consistent with linkage of idiotype

and heavy-chain allotype. All mice lacking the A/J allotype failed to produce the idiotype characteristic of anti-Ar antibodies of the A strain, whereas 19 of 21 allotype-positive offspring produced substantial amounts of the idiotype and the other two mice produced small but detectable quantities. These results are reminiscent of those observed with F_1 mice.

Quite different results were obtained when the second (non-A/J) strain was PL/J. This is one of four strains shown by Gottlieb and Edelman to possess a unique V_κ peptide which can be used as a genetic marker. The gene controlling this light-chain allotype is closely linked to the *Ly-3* locus. All offspring of the mating $(A/J \times PL)F_1 \times PL$, which lacked the A/J heavy-chain allotype, failed to produce the anti-Ar idiotype. In addition, however, five of eight offspring which were positive for the A/J allotype produced only small concentrations of the idiotype; three of the offspring were positive for both A/J allotype and the idiotype. Each of the mice that was positive for both allotype and idiotype was found to be heterozygous with respect to its Ly-3 phenotype (*i.e.*, Ly-3.1, 3.2), indicating that it was capable of synthesizing the light chain of both A/J and PL origin. The five mice which were positive for the A/J allotype but produced low concentrations of the idiotype were homozygous for Ly-3.1, indicating the absence of light chains of A/J origin. Thus, the production of the idiotype with this mating pair requires the presence of L chains of A/J origin. The results suggest that mice of the BALB/c, C57BL, and CBA strains can furnish L chains necessary for the expression of the idiotype, whereas mice of strain PL cannot. The presence of the unique allotypic marker in the PL strain may reflect the presence of a repertoire of L chains that differs from that of the majority of strains possessing the other V_κ allele. It should be of interest to ascertain whether the linkage between idiotype and heavy-chain allotype also breaks down when breeding is carried out with A/J mice and mice of the other three strains carrying the V_κ allele of the PL strain. It also seems possible, since these four strains have not previously been used in mating studies, that these findings might apply to other idiotypic systems as well.

Attempts were also made to produce the anti-Ar idiotype characteristic of the A strain by immunizing $(BALB/c \times NZB)F_1$ or $(BALB/c \times AKR)F_1$ mice against the Ar hapten group. The rationale was that BALB/c mice can probably provide the required light chains, as shown by the production of the idiotype by C.AL-20 mice, which possess the allotype of the AL/N strain on a BALB/c background; and that the other two strains have heavy-chain allotypes similar or identical to those of strains A/J or AL/N, both of which are idiotype-positive. It was therefore conceivable that the NZB or AKR strains might provide the necessary heavy chain. However, upon immunization, both of the F_1 strains produced Ar antibodies lacking the idiotype. This suggests that the V_H repertoire of the NZB and AKR strains may differ from that of the A strain.

Response of a Variant Strain of Rabbits (Basilea) to the Streptococcal Group A-Variant Polysaccharide. D.G. Braun, A.S. Kelus, and S. Weiss. Basel Institute for Immunology, Basel, Switzerland.

A variant line of rabbit, *Basilea*, which is homozygous for *bas/bas* light chains of the λ type, was hyperimmunized with a streptococcal group A-variant vaccine (A486 var.). Homozygotes *bas/bas* produced antibodies with λ chains; heterozygotes, however, produced predominantly ($>95\%$) antibodies with κ chains. The incidence of restricted high responders in the *Basilea* line was high; this high frequency is probably the result of a "founder effect" rather than to the loss of κ chains. The degree of heterogeneity of *bas/bas* homozygotes in response to Av-CHO is similar to that of *b4/bas* heterozygotes as well as to that of usual rabbits expressing mainly κ chains. This suggests that the pool of λ-chain structural genes in rabbits is of a size similar to that of the pool of κ chain structural genes, and that expression is a regulatory event which is under the control of alleles (*b4,b5,b6,b9,bas*).

Multiple IF Phenotypes of Normal Mouse Light Chains. D. Gibson, H. Noel, and D. Cote, Département de Biochimie, Université de Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4.

The light chains of normal mouse serum immunoglobulins were analyzed by gel isoelectric focusing using an improved version of the method originally described (D. Gibson: *J. Exp. Med. 144*:298, 1976). The focusing pattern observed in the majority of inbred mouse strains was highly reproducible and was designated phenotype A. Strains so far found to express light-chain pattern A include A/HeJ, A/J, A.By/Sn, A/WySn, BALB/cJ, BUB/BnJ, CBA/J, CBA/caJ, CBA/H-T6J, C57Bl/KsJ, C57Bl/6J, C57bl/10J, C57Bl/10Sn, C57Br/cdJ, C57L/J, C3HeB/FeJ, DBA/1J, DBA/2J,

129/J, and LP/J. Strains AKR/J, PL/J, and RF/J shared identical light-chain focusing patterns designated phenotype B. This pattern was distinguishable from pattern A in the position of several focusing bands throughout the pH range. The differences between patterns A and B appeared to be more extensive than initially realized. A third phenotype (type C), has so far been found only in the light chains of C58/J mice. This pattern was closely related to phenotype B, but differed in the absence of at least two notable bands compared with phenotype B. A fourth phenotype (type D) was identified in the light chain patterns of BDP/J, CE/J, and NZB/BINJ mice. This pattern was closely related to pattern A but also possessed features found in pattern C. It may possibly represent an intermediate form between the two phenotypes (A and C). (Supported by the Medical Research Council MA-4317.)

Identification and Genetics of λ_1 Light-Chain Allotype in the Mouse. R. Lieberman, W. Humphrey, Jr., and C.C. Chien. National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014.

Allogeneic antisera prepared in SJL mice immunized separately with BALB/c myeloma proteins HOPC1 (λ_1 IgG_{2a}) and MOPC 104E (λ_1 IgM) were absorbed with LPC1 (k IgG_{2a}) and MOPC774 (k IgM), respectively, to remove antibodies to IgC_H regions. Both antisera were λ-specific and identified antigenic specificities on RPC20 (λ_1 B.J.), S5444 and HOPC1 (λ_1 IgG_{2a}), J558 (λ_1 IgA), and MOPC104E (λ_1 IgM), and not on kappa myeloma proteins (IgM, IgG_{2a}, IgG_{2b}, IgG1, and IgA). Distribution studies showed most strains tested (*e.g.*, BALB/c, C57BL/6, DBA/2, A/J, CE, etc.) possessed the λ marker designated λL^1 except for SJL, BSVS, YBR, DE, and SJA, which were λL^{-1}. The SJA mouse is congenic to SJL except for the BALB/c heavy chain. Immunoglobulin precipitated from 10 ml SJL serum and reconstituted in 1/10 its original volume was also negative for the λL^1 marker. Geckeler and coworkers (W. Geckeler, B. Blomberg, C. dePreval, and M. Cohn: *Cold Spring Harbor Symp. Quant. Biol.*, 1976) reported that regulatory or structural genes affected the V_λ; mice of the $V\lambda_1^{lo}$ type produced low levels of λ in their immunoglobulins (*e.g.*, SJL); mice of $V\lambda_1^{hi}$ type produced high levels of λ (*e.g.*, BALB/c). We were interested in studying the possible relationship of the λL^1 and $V\lambda_1^{hi}$ genes. Accordingly, we prepared xenogeneic antiserum specific for λ by immunizing a goat with MOPC 104E (λIgM) and absorbing the antiserum with M774 (kIgM) and RPC5 (kIgG_{2a}). Sera from strains that were either λL^1 or λL^{-1} were tested for levels of λ by using them as inhibitors of the goat anti-M104E serum reacted to RPC20 (λ_1 B.J.)-coated SRBC in an HI system. The level of λ in the serum was estimated by comparing the HI titer of a known amount of RPC20 that gave complete inhibition [300 μg gave an HI titer of 12 (log 2)] with the HI titer of sera from different strains that gave complete inhibition [*e.g.*, BALB/c gave an HI titer of 7 (log 2)]. BALB/c serum contained 9.37 μg/ml, SJL had 0.58 μg, and BSVS had 1.16 μg, respectively. The genetics of λL^1 and λ_1^{hi} were studied in a backcross and F_2 progeny of BALB/c ($\lambda L^1 V\lambda_1^{hi}$) and SJL ($\lambda L^{-1}$, $V\lambda_1^{lo}$). Their sera were examined for allotype, λL markers, and λ_1 levels. λL^1 allotype genes segregated independently of the IgC_H genes. BC progeny of a cross of BALB/c/SJL × SJL/SJL showed that 15 of 32 progeny had both BALB/c (a^1) and SJL (a^2) allotypes. Eight of these had λL^1 and seven were λL^{-1}. The λL^1 was associated with λ_1^{hi}; λL^{-1} was associated with λ_1^{lo}. F_2 progeny tended to have higher levels of λ associated with λL^{-1} than those found in BC progeny, suggesting that other genetic factors contributed to this system.

Linkage of Genes Controlling an L-Chain Marker (K-PC8) and Ly-3 Alloantigen. Latham Claflin, Benjamin A. Taylor, Marianna Cherry, and Marge Cubberley. Department of Microbiology, University of Michigan, Ann Arbor, Michigan 48109, and the Jackson Laboratories, Bar Harbor, Maine 04609.

Mouse strains genetically different at a variety of loci, including the MHC and IgCH allotype loci, all produce antibody to phosphorylcholine (PC), some of which bears idiotypic, structural, and functional characteristics of a PC-binding myeloma protein HOPC8 (TEPC15). A previous paper identified a genetic marker in the L chains of the H8-like antibodies. By isoelectric focusing, two phenotypes were observed: *K-PC8-A* (reference strain, AKR) and *K-PC8-B* (reference strain, C57L). The initial study indicated a genetic association between genes that determine K-PC8 and the T-lymphocyte surface antigen *Ly-3* in linkage group XI (chromosome 6). To assess this relationship, recombinant inbred (RI) lines and congenic strains were examined. Among 15 AKXL RI lines studied, there was complete concordance of *K-PC8-A* with *Ly-3,1* and of *K-PC8-B* with

Ly-3,2. AKXL lines 13, 24, 25, 28, and 29 (all *Ly-3,1*) were found to be *K-PC8-A*, and AKXL lines 4, 6, 8, 12, 16, 17, 21, 36, 37, and 38 were *K-PC8-B* and *Ly-3,2*. Further support for linkage of *K-PC8* and *Ly-3* was found in mice congenic to C57BL/6 (*K-PC8-B, Ly-3,2*) but carrying the $Ly\text{-}3^a$ locus of either RF or PL (both *K-PC8-A, Ly-3,1*). B6·$RF\text{-}Ly\text{-}2^aLy\text{-}3^a$ (derived by E.A. Boyse) and B6·PL-$Ly\text{-}2^aLy\text{-}3^a$/Cy (derived by M. Cherry) were found to express an L chain that contained the *K-PC8-A* marker. Continued backcrosses of the $Ly\text{-}3^a$ locus of PL onto B6 (N24) still show coinheritance of the $K\text{-}PC8^a$ locus. Thus, among 21 inbred strains, 15 RI lines, and two different congenic strains, there is complete concordance of *K-PC8-A* with *Ly-3,1* and of *K-PC8-B* with *Ly-3,2*. By contrast, *K-PC8* (*Ly-3*) and *Ig-1* segregated independently. Though no recombinants have been observed between *K-PC8* and *Ly-3*, the upper limit of the recombination frequency (r) can be estimated from the data on the RI lines and *Ly-3* congenics. A computed r at the 95 and 99 percent confidence levels are 0.016 and 0.02, respectively. While the most parsimonious view of the data postulates that the *K-PC8* polymorphism reflects variation in the V_κ library of different strains, the data is not inconsistent with the idea of a regulatory locus governing κ-chain expression which maps in linkage group XI.

Construction of Ly-2,3-Congenic Strains for the Study of Mouse L-Chain Genetic Polymorphisms. Paul D. Gottlieb. MIT Center for Cancer Research and Department of Biology, Cambridge, Massachussetts 02139.

We have constructed four new Ly-2,3-congenic mouse strains for use in studies of the I_B-peptide marker and other L-chain polymorphisms. In constructing each strain, cells from thymic biopsies of backcross progeny were assayed for the Ly-3 antigenic specificity of the donor strain. This mode of testing was chosen for its ease as compared with monitoring for the closely linked L-chain polymorphisms. The characteristics of each strain are listed in Table 3.

Pristane has been injected intraperitoneally into mice of the BAK-9 and BC58-8 strains to induce plasmacytomas. Myeloma proteins produced by those tumors will be screened for expression of the I_B-peptide marker and other L-chain polymorphisms linked to the $Ly\text{-}2^a$, $Ly\text{-}3^a$ gene complex. Structural studies of such proteins should allow detailed characterization of these polymorphisms. In addition, studies of the set of myeloma L chains obtained should reveal the extent to which the repertoire of V_L-regions governed by this L-chain-related locus if the BALB/Ly-2^a, Ly-3^a strains differs from that of their Ly-2^b, Ly-3^b, I_B-negative congenic partner, BALB/c (see Nisonoff, these abstracts). (This work was supported in part by U.S. Public Health Service Research Grant CA 15808 from the National Cancer Institute to P.D.G., Grant #IM-113 from the American Cancer Society to P.D.G., and by U.S. Public Health Service Research Grant CA 14051 from the National Cancer Institute to the MIT Center for Cancer Research.)

Diversity of L Chains in Murine Antistreptococcal Antibodies. R.M. Perlmutter, D.E. Briles, and J.M. Davie. Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63130.

Table 3. Ly-2,3-Congenic Strains

Strain Abbreviation	Strain	Phenotype		Inbreeding Generation	Further Backcross Generation
		Ly-3	I_B		
BAK-9	BALB/AKR-Ly-2^a, Ly-3^a	Ly-3.1	(+)[b]	N9F3	N12
BC58-8	BALB/C58-Ly-2^a, Ly-3^a	Ly-3.1	(+)[b]	N8F3	N13
AKBA-10	AKR/BALB-Ly-2^b, Ly-3^b	Ly-3.2	(−)[b]	N10F3	N12
C58B[a]	C58/BALB-Ly-2^b, Ly-3^b	(Ly-3.2)[c]	(−)[b]	–	N10

[a] Strain not yet inbred
[b] These are expected phenotypes. Testing for the I_B-peptide marker and for other Ly-3-linked L-chain polymorphisms is in progress
[c] Phenotype after inbreeding will be Ly-3.2

To examine the diversity of antibody light chains, we have developed an analytical isoelectric focusing procedure which permits the routine analysis of L chains from antibodies raised in individual mice. We have used this technique to demonstrate that:

1) IgM and IgG anti-group A streptococcal antibodies raised in SWR mice share identical L-chain spectrotypes;

2) murine antistreptococcal antibodies exhibit a strain-determined variation in the degree of heterogeneity of their constituent L chains;

3) different mouse strains also vary in the frequency with which they express particular L-chain spectrotypes; and

4) multiple antibodies of identical H-chain isotype but with distinguishable isoelectric points share identical L-chain spectrotypes.

These results underline the importance of combinatorial diversity in the elaboration of heterogeneous antibodies.

Structural Studies on Induced Antibodies with Defined Idiotypic Specificities. J. Donald Capra, Amar S. Tung, and Alfred Nisonoff. The University of Texas Health Science Center, Dallas, Texas 75235, and Rosensteil Research Center, Brandeis University, Waltham, Massachusetts 02154.

The complete amino acid sequence of the variable regions of light chains derived from anti-*p*-azophenylarsonate antibodies from A/J mice bearing a crossreactive idiotype is reported. At least two, and probably more than three, distinct light chains are associated with this idiotypically characterized antibody. The antibodies have several differences in their framework structures, but evidence is presented indicating that all three light-chain hypervariable regions have a homogeneous sequence. The data are discussed in relation to the various theories of antibody diversity. In addition, the findings support the view that hypervariable regions, idiotypic determinants, and the antibody combining site involve, to a large extent, the same molecular structures.

Amino Acid Sequence Comparison of Nine Structurally Related Mouse Kappa Ig Variable Regions. D.J. McKean and M. Potter. The Mayo Clinic, Rochester, Minnesota 55901, and Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland 20014.

A comparison of the amino terminal 23 residue amino acid sequence of BALB/c mouse kappa light-chain variable regions (V_κ) has shown these sequences to be heterogeneous. Fifty-three of 78 published mouse V_κ sequences have different amino terminal sequences (E.A. Kabat, T.T. Wu, and H. Bilofsky: *Variable Regions of Immunoglobulin Chains.* Medical Computer Systems, Cambridge, 1976). V_κ regions which have been completely sequenced show that this amino terminal sequence diversity extends throughout the entire variable region.

The variation pattern in V_κ regions which have nearly identical amino terminal 23 residue sequences was approached in an earlier study (D.J. McKean, M. Potter, and L. Hood: *Biochemistry 12*:760, 1973). The V-region sequences of three kappa chains (M321, M63, T124) were compared to a fourth V_κ region (M70) (W.R. Gray, W.J. Dreyer, and L. Hood: *Science 155*:465, 1967). This initial comparison has now been extended to five additional V_κ regions which have similar amino terminal sequences.

These five M321-like V_κ regions were identified in a screening of myeloma tumors with idiotypic antisera specific for the M321-like V_κ regions (Weigert and Julius, personal communication; McKean and Potter, unpublished data). Five additional myeloma proteins were identified in these screenings, but have not been sequenced.

The partial amino acid sequence of four BALB/c and one C57/BL V_κ chains are compared with the four M321-like V_κ regions previously published (Table 4). The V_κ sequences can be classed into at least five groups on the basis of linked amino acid substitutions. These include M321, T124; M63, AB22, T62; M70, B32; B61; and BPC-1. Although many of these sequences are incomplete, the substitutions within each of these groups have so far occurred only within the complementarity regions.

It is hoped that additional sequence comparison of other M321-like proteins will facilitate our understanding of the mechanisms of antibody diversity. The M321-like kappa chains present a unique system for comparing theories of antibody diversity as interpreted from amino acid sequence data with the numbers of V_κ genes as determined by nucleic acid hybridization techniques.

Table 4. Amino Acid Substitutions Among V_κ Regions with Similar Amino Terminal 23 Residue Sequences

Light Chains Compared	Amino Acid Differences			Total Residues Compared[a]
	Total	Complementary	Framework	
M70-M321, M63, T124	20–21	8–9	9–11	111
M63-M321, T124	10	4	6	111
M321-T124	3	3	0	111
M63-AB22	1	1	0	106
M63-T62	0	0	0	45
M70-B32	6	6	0	89
B61-M321, M63, T124	1	N.D.[c]	1	24
BPC-1[b]-M321, M63, T124	5–8	5–7	0–1	40

[a] V_κ region is comprised of 111 residues
[b] BPC-1 is of C57BL origin
[c] N.D. = no hypervariable regions have been sequenced

Primary Structure of Galactan-Binding Myeloma Proteins. D.N. Rao, Stuart Rudikoff, and Michael Potter. National Institutes of Health, Bethesda, Maryland 20014.

The primary structure of a group of galactan-binding proteins is being determined to assess the pattern of variation and to compare this pattern with that previously observed in antiphosphyryl-choline proteins. To date, two light chains are essentially complete and a third lacks about 20 amino acids, including L2. Seven substitutions are found among the three chains, five of which are located in complementarity regions. The substitutions occurring in the framework are both found at residue 100, resulting in all three chains having a different amino acid at this position. Six of the seven substitutions can be accounted for by single-base nucleotide changes.

Partial sequences of the heavy chains have been obtained for positions 1 to 40 and 83 to 120, which includes H1 and H3. For the heavy chains from J-539 and X-44, only two amino acid interchanges are found, both of which are in complementarity regions and one of which would require a two-base nucleotide change. The third chain, X-24, differs from H-539 at eight positions, three of which occur in complementarity regions and five in the framework. Two of these interchanges would require two-base nucleotide changes. Additionally, three of the substitutions located near the switch region suggest the possible occurrence of insertions and deletions of nucleotide triplets, resulting in a shift in position of apparently linked amino acids but not causing a frameshift in the DNA readout.

Control Over Expression of λ_1Chain. William Geckeler and Melvin Cohn. The Salk Institute, La Jolla, California 92037.

The λ_1 light chain in mice is encoded by a single gene for the variable region (v_{λ_1}) and a single gene for the constant region (c_{λ_1}). The λ_1 light chain class typically represents about one percent of the total normal light chain in most inbred mouse strains. In several inbred strains, the amount of λ_1 light chain associated with normal immunoglobulin is reduced fiftyfold. This abstract concerns the genetic control of λ_1 light chain expression.

The genetic control of the amount of λ_1 light chain associated with normal immunoglobulin was studied by mating an inbred strain with high λ_1 levels to one with low λ_1 levels. The λ_1 levels in the first three filial generations of this mating were quantitated. The results suggest that two alleles, λ_1^+ and λ_1^{10}, at a single genetic locus, λ_1, control λ_1 expression. The λ_1 locus is linked to the λ_1 structural gene locus, since F_1 progeny have an amount of λ_1-bearing immunoglobulin intermediate to both parental strains. The distribution of the λ_1^+ and λ_1^{10} alleles in the F_2 generation indicates that the λ_1 locus is unlinked to either of the two genetic loci at which antigen-specific immune response genes have been mapped, the major histocompatibility complex of the heavy-chain gene complex.

The mechanism by which the λ_1 locus regulates λ_1 light chain expression was studied further in several ways. First, splenic antigen-sensitive B cells (ASCs) from λ_1^+ or λ_1^{10} homozygotes were stained for their light-chain class with fluorescently labeled anti-λ_1 or anti-λ antibodies. The results indicate that λ_1^{10} homozygotes have a fiftyfold reduction in the number of λ_1-bearing ASCs compared with λ_1^+ homozygotes. $\lambda_1^+/\lambda_1^{10}$ heterozygotes have one-half the number of λ_1-bearing ASCs as do λ_1^+ homozygotes. Second, the λ-1,3 dextran antibody response in $\lambda_1^+ v_{H\lambda_1}^{\alpha-1,3}$ or $\lambda_1^{10} v_{H\lambda_1}^{\alpha-1,3}$ homozygotes was examined. $\lambda_1^+ v_{H\lambda_1}^{\alpha-1,3}$ homozygotes are known to produce a high-magnitude α-1,3 dextran antibody response in the λ_1 light-chain class, regardless of the remaining genetic background. $\lambda_1^{10} v_{H\lambda_1}^{\alpha-1,3}$ homozygotes produce a λ_1-bearing α-1,3 dextran antibody response, the magnitude of which varies from unresponsiveness to high responsiveness. The individual variation in the response magnitude is produced by a random process, since progeny of nonresponder matings or high responder matings show the same wide variation in responsiveness. The variation in responsiveness in $\lambda_1^{10} v_{H\lambda_1}^{\alpha-1,3}$ homozygotes follows a Poisson distribution for which the average number of responding units is 1.6. The response variation for $\lambda_1^+ v_{H\lambda_1}^{\alpha-1,3}$ homozygotes suggest that they have an average number of responding units greater than or equal to 4.6.

The alleles of the λ_1 locus act before the ASC stage by a mechanism which is subject to allelic exclusion, since $\lambda_1^+/\lambda_1^{10}$ heterozygotes have one-half the number of ASCs and one-half the level of λ_1-bearing normal immunoglobulin as do λ_1^+ homozygotes. The alleles of the λ_1 locus do not appear to be alleles of the v_{λ_1} or c_{λ_1} structural gene, since peptide fingerprints of the λ_1 light chains produced by λ_1^+ and λ_1^{10} homozygotes are indistinguishable. Instead, the alleles appear to be truly regulatory and to affect the probability that a particular ASC precursor committed to λ_1 light-chain expression will differentiate successfully. The λ_1^+ and λ_1^{10} alleles might, for instance, represent a single base pair difference in a DNA sequence involved in v_{λ_1}-c_{λ_1} translocation. If such a difference affects the efficiency of the translocation process, all the observed phenotypic effects of the alleles at the λ_1 locus are explicable.

Structural and Serological Analysis of the V_κ21 Isotope. M.A. Julius, J. Gatmaitan, and M. Weigert. The Institute for Cancer Research, Philadelphia, Pennsylvania 19111.

A high frequency ($\sim$10%) of BALB/c and NZB myeloma kappa chains have identical sequence up to the first cysteine. This group of kappa chains has been designated the V_κ21 isotope (summary, Table 1). The complete sequence of several BALB/c cases (McKean *et al.*, *Biochem. 12*:760, 1973) shows that these light chains subsequently diverge in framework sequence. Therefore, by a stringent definition of an isotype, V_κ21 must be divided into two isotypes. These have been designated V_κ21A (prototype sequence M70E) and V_κ21C (prototype sequence M321). Two additional BALB/c kappa chains, M63 and ABPC22, have an N-terminal sequence similar to that of the V_κ21 isotype, although they do not have the N-terminal Asp, which is present in all members of V_κ21A and C. Instead, Asp has been replaced by Asn. M63 and APBC22 comprise a separate isotype, V_κ21B.

Preliminary sequence data indicate that NZB has several V_κ21A homologs (PC1229, PC2880, PC7132) and at least one representative of V_κ21C (PC3741). To investigate more fully the pattern of variation in the NZB equivalent to V_κ21, we have prepared heterologous antisera against two V_κ21 light chains, PC3741 (V_κ21C) and PC2880 (V_κ21A), according to the method of Potter and McKean (personal communication). Competitive radioimmunoassays demonstrate that PC3741 and PC2880 react specifically with their respective antisera, and equivalent competition is obtained with the homologous light chain or with the intact immunoglobulin. Hence, the V_κ21A and C isotypes each possess a variable-region determinant which apparently remains unmodified and exposed to solvent even in the presence of heavy chain. The ability of these antisera to recognize light-chain isotypes in the intact immunoglobulin has enabled us to identify additional examples of the V_κ21 isotype by screening myeloma ascitic fluids directly. To date, 11 examples bearing the V_κ21C determinant and 27 examples bearing the V_κ21A determinant have been identified. The frequency of myeloma proteins bearing V_κ21C and A determinants is three and seven percent, respectively.

These isotypes can be detected in the normal serum of NZB. The percentage of normal kappa-bearing V_κ21A and C determinants is equal to the percentage of myeloma proteins which type positively with the heterologous antisera. Four other strains examined (BALB/c, C57BL/6J, A/He, and C58) have levels equivalent to NZB, but rat serum was found to be negative. Thus, it appears that the V_κ21 isotype is present in many, if not all, mouse strains and is species-specific.

The most extensively examined isotype of NZB is V_κ21A. So far, for this subgroup, two

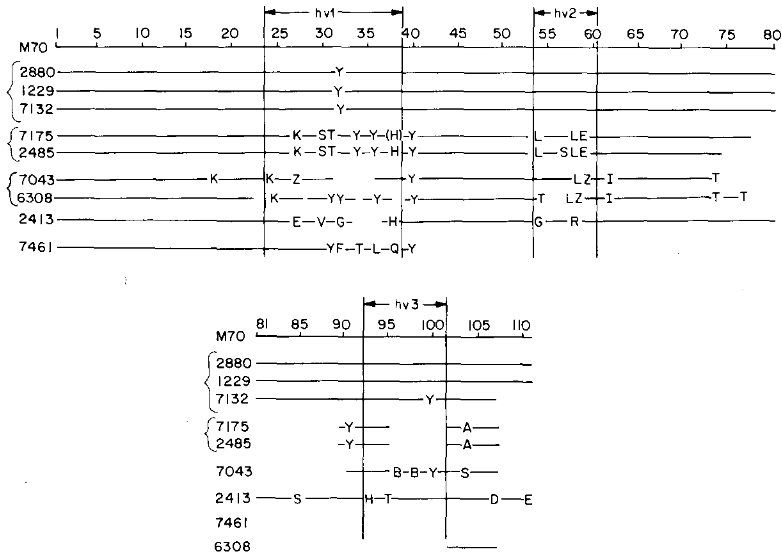

Fig. 1. The partial amino acid sequences of M70-like NZB V_κ regions. These V_κ sequences have been designated M70-like because of their similarity to a previously sequenced BALB/c κ chain, M70. Brackets indicate subsets of proteins. Some of the data in this figure are derived from homology relationships between the amino acid compositions of peptides from different proteins

identical sequences (PC1229 and PC2880) and one variant (PC7132) have been found. The variant has two Trp→Tyr substitutions at positions 28 and 96. Hence V_κ21A exhibits a pattern of variation analogous to mouse V_λ. Additional isotypes have been identified, the sequences of which are identical to the V_κ21 isotype up to residue 23, but subsequently diverge. This suggests that there may be many isotypes homologous to V_κ21A, B, and C. Alternatively, the number of isotypes may be low, and variations in framework may reflect somatic variation.

Amino Acid Sequence Analysis of a Set of Closely Related Chains From Mouse. E. Loh [1], J. Schilling [1], J. Gatmaitan [2], M.A. Julius [2], M. Weigert [2], and L. Hood [1]. [1] Division of Biology, California Institute of Technology, Pasadena, California 91125, and [2] The Institute for Cancer Research, Philadelphia, Pennsylvania 19111.

The sequence analysis of sets of closely related variable (V) regions has placed important constraints on thinking about theories of antibody diversity and the organization of antibody structural genes. We have found a set of NZB myeloma proteins which is closely homologous to a BALB/c myeloma κ chain, MOPC 70. This set is one of the most promising sets of closely related V sequences for examining diversity patterns, because approximately ten percent of NZB myeloma κ chains fall into this set. Moreover, specific serological reagents have been prepared to facilitate the identification of members of this set. Thus, a large number of V_κ regions is available for protein or nuclei acid analysis.

The partial amino acid sequences from nine V_κ regions of this M70-like set have been examined (Fig. 1). Three important observations emerge. First, two of nine sequences are identical (2880 and 1229), while the remainder differ by from one to 14 residues from one another. Thus, almost every V_κ sequence differs from all the others. Second, substitutions occur in framework, as well as hypervariable regions. Third, there are three subsets (2880–1229–7132, 7175–2485, and 7043–6308) which share identical amino acid substitutions in framework, as well as hypervariable regions. These patterns of sequence diversity already begin to place important constraints on theories of antibody diversity (L. Hood, M. Kronenberg, P. Early, and N. Johnson: Nucleic acid chemistry and the antibody problem. *In* E.E. Sercarz, L.A. Herzenberg, and C.F. Fox (eds.): *The Immune*

System II: Regulatory Genetics. Academic Press, New York, in press, 1977). The amino acid sequence and serological studies now in progress on additional proteins from this set should provide important insights into the nature of diversity among closely related V regions.

Ig-Dependent Helper T Cells. C.A. Janeway, Jr., R. Asofsky, F. Weinbaum, R. Murgita, and H. Wigzell. Yale University School of Medicine, New Haven, Connecticut 06510, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014, and Uppsala University, Uppsala, Sweden.

It has been demonstrated that carrier-primed helper T cells, when assayed in an adoptive secondary antihapten antibody response, behave as though they contained two interacting cell populations. This is detected by the slope of the log antibody response as a function of the log carrier-primed spleen cells transferred. Early after boosting, the slope of this straight line approximates two. This behavior is not affected by adult thymectomy, and is stable from six days to six months after carrier priming. Therefore, both activities are thought to result from mature, recirculating T cells. Both activities can be shown to depend on carrier priming. Both are equally sensitive to treatment of the cell donor with antithymocyte serum. In the present experiments, it has been shown that carrier-primed spleen cells passed over anti-immunoglobulin columns to remove all B cells still retain full helper activity, and still show log dose response curves with slopes of two. On the other hand, helper T cells derived from carrier-primed donors that were treated from birth with antimouse μ chain antibody give decreased helper activity, and their log dose response curve has a slope slightly less than one. Thus, helper T cells raised in an agammaglobulinemic environment appear to lack one of the postulated T helper cells. This conclusion is reinforced by the finding that such cells, when added to carrier-primed spleen cells from normal control mice give three times as much antibody as would be predicted by simple addition of the activity of each population taken alone, that is, the two populations can be shown to synergize. Because both cell populations pass through anti-immunoglobulin columns intact, surface immunoglobulin on the helper T cell is not thought to be important in their function. Rather, it seems likely that such helper T cells are dependent on the presence of immunoglobulin for their priming by carrier. It is possible that there are two types of helper T cell differing in their specificity, one recognizing carrier determinants in association with Ia antigens and the other recognizing carrier determinants in association with immunoglobulin.

Hapten-Binding Receptor Molecules Isolate From Nylon Wool-Enriched T Lymphocytes. M. Cramer U. Krawinkel, T. Imanishi-Kari, R.S. Jack, and K. Rajewsky. Institute for Genetics, University of Cologne, Weyertal 121, D-5000 Köln 41, Federal Republic of Germany.

Hapten-specific receptor material isolated from sensitized mouse lymphocytes can be separated into a fraction expressing serological determinants of immunoglobulin (anti-Ig^+ fraction) and a fraction which lacks determinants of immunoglobulin-constant regions, including those of kappa and lambda light chains (anti-Ig^- fraction) (U. Krawinkel and K. Rajewsky: *Eur. J. Immunol. 6*:529, 1976). Experiments using enriched B- and T-cell populations suggest that the anti-Ig^+ fraction is B cell-derived, whereas the anti-Ig^- fraction originates from T cells. The anti-Ig^- fraction of 4-hydroxy-3-nitro-phenylacetyl (NP)-specific receptor material obtained from C57BL/6 mice was found to carry two markers which, at the level of primary anti-NP antibodies of C57BL/6 mice, define immunoglobulin heavy-chain variable regions. These markers are the characteristic fine specificity of hapten-binding (heteroclicity) (T. Imanishi and O. Mäkelä: *J. Exp. Med. 140*:1498, 1974) and an idiotypic marker defined by an anti-idiotypic antiserum raised against primary anti-NP antibodies. Both markers are absent in NP-specific anti-Ig^- receptor material derived from CBA mice. In genetic experiments the two markers segregate with the Ig-1^b allotype at both the level of humoral antibodies and of the anti-Ig^- receptor fraction. These results suggest that the receptor molecules of the anti-Ig^- fraction share with antibodies the variable portion of immunoglobulin heavy chains. Results with receptor molecules of rabbit origin support this interpretation. Here again, hapten-binding material can be isolated from enriched T-cell populations, and these molecules lack determinants of immunoglobulin-constant domains but express the *a*-locus allotype.

Regulation of Expression of p-azopheylarsonate Antibodies. A. Nisonoff and F.L. Owen. Rosensteil Research Center, Brandeis University, Waltham, Massachusetts 02154.

Pretreatment of adult A/J mice with rabbit anti-idiotypic antisera prevents the appearance

of the idiotype associated with antibodies to the *p*-azophenylarsonate (Ar) group that is otherwise produced by all members of that strain. When such suppressed mice are hyperimmunized, they produce high titers of anti-Ar antibody lacking the idiotype. It was found that, if eight to 12 weeks are allowed to elapse after suppression and hyperimmunization, such mice possess large proportions of splenic T cells with specificity for the idiotype. This was shown by rosetting experiments in which autologous RBC were coated with Fab fragments of anti-Ar antibodies possessing the idiotype. The rosette-forming splenic lymphocytes were found to be sensitive to anti-Thy-1.2 plus complement, and to be capable of resynthesizing the receptors after treatment with trypsin. Only small proportions of rosette-forming cells were elicited in animals that were suppressed with respect to the idiotype but not subsequently immunized. The mechanism by which antigen induces the formation of large numbers of idiotype-specific T cells is not known.

It was found that the rosette-forming T-cell population includes the idiotype-specific suppressor T cells, first identified by Eichmann in another system. Evidence for this was the depletion of suppressor cells by removal of rosettes and the presence of a high level of suppressor activity in those cells which formed rosettes. These observations should permit a study of factors influencing the production of idiotype-specific suppressor T cells and, possibly, of soluble suppressor factors that may be derived from these large numbers of idiotype-specific cells.

Allotype-Dependent Immunoregulation of Primary B-Cell Responses. S.K. Pierce. University of Pennsylvania, Philadelphia, Pennsylvania 19104.

Much evidence has accumulated which demonstrates that an animal's immune system has the capacity to recognize its own antibody idiotypes. These findings suggest that self idiotypic recognition may potentially play a role in the regulation of B-cell responses. The experiments presented in this report were carried out to determine if an animal develops the ability to regulate specifically the synthesis of antibodies, specific for an antigen, subsequent to primary immunization to the particular antigen and concomitant with an initial antibody response. Employing the splenic fragment culture system, we have compared the response of primary donor B cells in irradiated recipients which have been immunized previously to Hy alone or Hy and DNP-Hy. The results indicated that only 25 to 30 percent of DNP-specific B-cells stimulated by DNP-Hy in Hy-immunized recipients could be stimulated by DNP-Hy in recipients immunized with Hy as well as DNP-Hy. B-cell responses to other haptens, such as FL-Hy, and secondary DNP-specific B-cell responses were unaffected in DNP-Hy-immunized animals. The nontrivial and specific nature of the observed decrease in primary DNP-specific B-cell responses was verified by the finding that the response of CB20 donor cells, which differ from BALB/c mice only in the immunoglobulin heavy-chain allotype-linked locus, was unaffected in BALB/c recipient mice which had been immunized with DNP-Hy. Thus, it appears that during a primary humoral immune response to a T-dependent antigen, a regulatory mechanism is induced which specifically limits the stimulation of hapten-specific primary, but not secondary, B cells. These studies have had practical value in providing a means for obtaining carrier-specific help for antigens for which carrier molecules are not readily available, such as viruses and bacteria. The important implications these findings have for the understanding of the control of primary B-cell responses and the generation of secondary B cells was discussed.

Disproportionate Representation of IgG_1 and IgG_{2a} in Ig-Congenic BALB/c Mice. M.J. Bosma and J. Owen. The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.

BALB/c · Ig^b-congenic mice (C.B mice) carrying C_H allotype genes of C57BL origin (from C57BL/K_a mice) produce from six- to tenfold less IgG_{2a} than C57BL/6 mice of the same age (20–30 weeks), whereas IgG_1 production at this time is equivalent in both mouse strains. This is shown in Figure 2. Here, the serum concentrations of IgG_1 and IgG_{2a} allotypes in C57BL/6 and C.B-31 mice are plotted as a function of age. Although IgG_1 comes up faster in C57BL/6 than in C.B-31 mice (Fig. 2a and 2b), both strains show comparable IgG_1 concentrations at 20 to 30 weeks of age. This is in contrast to the age-dependent production of IgG_{2a} in both strains. In C57BL/6 mice, near-maximum levels of IgG_{2a} ($\sim$700 μg/ml) are seen six weeks after birth (Fig. 2a). On the other hand, IgG_{2a} levels in most C.B-31 mice rise to 400 to 500 μg/ml at eight weeks of age and then decline to $\sim$100 μg/ml by 20 weeks of age (Fig. 2). We have observed the same pattern of age-dependent changes of IgG_1 and IgG_{2a} in C.B-17 and C.B-26 mice. In some individual C.B mice, the rise and fall of IgG_{2a} production is very dramatic. For example, we have detected

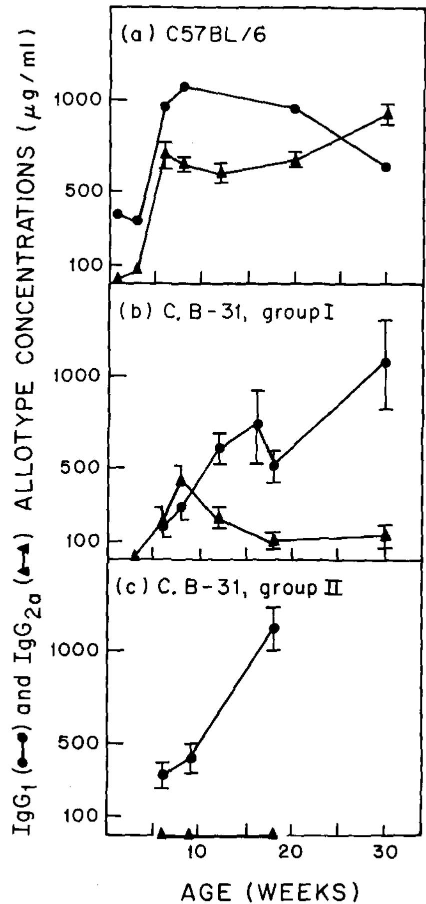

Fig. 2. Serum IgG_1 and IgG_{2a} allotype concentrations of C57BL/6 and C.B-31 mice as a function of age. (a) IgG_1 and IgG_{2a} levels in C57BL/6 mice; the points for IgG_{2a} represent the mean value for 12 mice, while the IgG_1 values were determined from a pool of the same samples; (b) mean values of IgG_1 and IgG_{2a} in 14-28 C.B-31 mice; and (c) IgG_1 and IgG_{2a} levels in nine unusual C.B-31 mice (see text). The same C.B-31 mice were used for all IgG_1 and IgG_{2a} determinations. IgG_1 and IgG_{2a} allotypes were quantitated by a radioimmune assay using the myeloma proteins M300 (IgG_1) and CBPC 101 (IgG_{2a}) as standards. The standard error of the mean is indicated by the bars

as much as 900 to 1300 μg/ml of IgG_{2a} in a few eight- to 15-week-old C.B-31 mice; the same mice have consistently shown less than 150 μg/ml of IgG_{2a} in subsequent weeks. At the other extreme, we have observed C.B-31 mice with no detectable IgG_{2a} for varying periods of time. This is illustrated for two unusual litters of C.B-31 mice in Figure 2c. Under the assay conditions used, none of these mice could be shown to produce IgG_{2a} (≤ 10 μg/ml). However, the same mice at 18 weeks of age are seen to be producing high levels of IgG_1 (~ 1000 μg/ml).

In summary, C.B. mice show a sharp rise and fall in their production of IgG_{2a} with increasing age, whereas their IgG_1 production continues to rise during the same interval of time (3–30 weeks of age). It is not yet clear to us why the BALB/c genetic background of C.B mice should have a negative influence only on the production of IgG_{2a}. However, one explanation is that this reflects allotype-specific suppression. Regulation of this kind could be mediated by varying numbers of cytotoxic or suppressor T cells specific for C57BL IgG_{2a} allotype in B cells. What makes this a reasonable possibility is our recent demonstration of such T cells in BALB/c mice (*J. Exp. Med.* *145*:743, 1977). (Supported by USPHS grants AI-13323, CA-04946, CA-06927, and RR-05539 from the National Institutes of Health, and by an appropriation from the Commonwealth of Pennsylvania.)

Evidence for Clonally Restricted T-B Interactions. H. Cantor and K. Ward. Department of Medicine, Harvard Medical School, Cambridge, Massachusetts 02138.

We have asked whether B cells carrying the crossreactive idiotype (A. Nisonoff and S.A. Bangassar: *Transplant. Rev.* 27:100, 1975) associated with the antiarsonate antibodies in A/J mice (CRI^+ B cells) are induced mainly by T cells carrying idiotypically related receptors. We have found that when CRI^+ B cells are induced by T_H cells from carrier-primed donors, these T_H cells can be eliminated by pretreatment of the donor with rabbit anti-CRI antibodies. We have not yet investigated the mechanism of T_H elimination after in vivo administration of anti-CRI, but we suspect that it reflects preferential induction of CRI-specific T_S cells, rather than a direct effect of anti-CRI on T_H cells. In either instance, these experiments indicate that induction of CRI^+ B-cell clones appears to be governed by idiotypically related T_H and T_S cells. The precision of this circuit may depend on complementary or cognate T_H surface structures.

It is possible that induction of other B-cell clones programmed to express dominant or germline V_H structures may be regulated similarly. We are currently testing the hypothesis that germline dominant antibodies are secreted by a specialized B-cell subclass, (*e.g.*, absent in CBA/N mutant mice) and that this B-cell subclass (and therefore this set of antibodies) is under stringent and highly specific T-cell regulatory control. According to this idea, the expression or nonexpression of germline antibodies, as defined, for example, by anti-idiotypic reagents, is due mainly to the presence or absence of regulatory genes governing differentiation of individual clones of $idiotype^+$ B cells, rather than by the presence or absence of structural genes required for synthesis of the appropriate V_H structures.

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