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Linkage in Mice of Genes Controlling an Immunoglobulin Kappa-Chain Marker and the Surface Alloantigen Ly-3 on T Lymphocytes*

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Abstract. Evidence obtained using recombinant inbred and congenic mouse strains has shown that the *PC8* locus responsible for determining a marker on a single κ chain in inbred mice is linked to the *Ly-2,3* locus on chromosome 6. The upper limit of the map distance between these loci is approximately three centimorgans. This finding is discussed in relation to other known light-chain variants that are associated with the *Ly-2,3* locus.

Introduction

In a previous paper (Claflin 1976a), a genetic marker was identified in the light (L) chain of antibody of a predominant idiotype in the response to phosphocholine (PC) in mice. The marker, designated PC8 [formerly designated KPC8 (Claflin 1976a)], is located in the κ chain in those anti-PC antibodies which show the same functional and idiotypic characteristics as a PC-binding myeloma protein, HOPC 8 (H8; Claflin 1976b). By analytical isoelectric focusing (IEF), two phenotypes were observed: PC8-A (reference strain, AKR/J) and PC8-B (reference strain, C57L/J). Light chains from other idiotypically identical anti-PC antibodies cofocus, supporting the idea that the PC8 marker resides in the variable, rather than the constant region (Claflin 1976a).

In the initial study, the strain distribution pattern of the PC8 phenotypes suggested a genetic relationship with the gene(s) that determine the Ly-2,3 lymphocyte antigens. The Ly-2 and Ly-3 alloantigens are part of a glycoprotein complex (Durda and Gottlieb 1976) expressed on the surface of thymus-derived suppressor (Jandinski *et al.* 1976) and cytotoxic (Cantor and Boyse 1975) lymphocytes. Since genetic (Itakura *et al.* 1972) and structural (Durda and Gottlieb 1976) data show that Ly-2 and Ly-3 antigens probably reside on a single protein, we will refer to a single locus, *Ly-2,3*, controlling specificities Ly-2 and Ly-3. The genetic locus is found on chromosome 6 (Itakura *et al.* 1972). Because PC8 is a marker on an L chain derived from what appears to be a single clonotype (Claflin 1976b), and since the phenotypic differences are readily identified, we were able to establish whether a relationship existed between genes controlling expression of Ly-3 alloantigens and mouse L chains. In the present investigation, recombinant inbred (RI) and congenic

* Abbreviations used in this paper are as follows: L, light chains; PC, phosphocholine; H8, HOPC 8; IEF, isoelectric focusing; KLH, keyhole limpet hemocyanin; RI, recombinant inbred

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lines of mice are used to demonstrate close linkage between the light chain IEF marker and the *Ly-2,3* locus.

Materials and Methods

Mice. Inbred mouse strains were obtained from the Jackson Laboratory, Bar Harbor, Maine. The AKXL RI strains of mice were developed by Taylor from successive brother-sister inbreeding from the F_2 generation of the AKR/J × C57L/J cross (Taylor and Meier 1976). Certain of the RI strains are reproducing poorly and, therefore, progeny of the outcross to C57L/J were analyzed. These included (C57L/J × AKXL-16)F_1, (C57L/J × AKXL-28)F_1, and (C57L/J × AKXL-29)F_1. Two *Ly-2,3* congenic strains, both on the C57BL/6 background, were used. The B6.PL-*Ly-2^a,Ly-3^a* congenic strain was developed by Cherry, who introduced the *Ly-2^a,3^a* allele of strain PL/J (detected in antibody-mediated cytotoxicity assays) onto the C57BL/6J background. At N11 some backcross mice were sib mated and *Ly-2^a,3^a* homozygous progeny were mated to form the congenic strain. Other N11 mice were mated to C57BL/6J to continue the backcross regimen, and mice of the 23rd backcross generation (N24) were also studied. The other congenic strain, B6.RF-*Ly-2^a,Ly-3^a*/Boy (Boyse *et al.* 1971), was a generous gift of Dr. E.A. Boyse of the Sloan-Kettering Cancer Center, New York. LT/Sv is an RI strain from the cross C58 × BALB/c and HP/Ei is an RI strain developed from the cross AKR/J × C57BL/6J (Taylor, unpublished data).

Antisera. Mouse IgC_H allotypes a^1 and a^2 were detected with C57BL/6 anti-BALB/c and BALB/c anti-C57Bl/6 antisera, respectively. The antisera were produced by immunization with washed pertussis anti-pertussis agglutinates (Potter and Lieberman 1967). Typing for a^1 and a^2 determinants was determined by micro-Ouchterlony immunodiffusion. Mice of the AKXL lines were typed for *H-2* (lymph nodes) and *Ly-2,3* (thymocytes) by a ^{51}Cr-release antibody-mediated cytotoxic assay (Snell *et al.* 1971). The alloantisera used are listed in Table 1. All were operationally monospecific in the AKXL system except for AS-523, which was absorbed with thymocytes of strain C57L to remove a minor unidentified specificity.

Purification of Anti-PC Antibodies. Antisera were obtained from mice 4 and 6 days after each of 2 bi-weekly injections of 10^8 heat-killed (56°C, 60 minutes) *Streptococcus pneumoniae* strain R36A (Claflin and Davie 1974). Serum from successive bleedings of 4–7 mice (inbred strains) or individual mice (F_1) were pooled and anti-PC antibodies purified by affinity chromatography as previously described (Claflin 1976b). Sera from BSVS mice were obtained from Dr. Briles, Washington University School of Medicine, St. Louis. In some experiments, anti-PC antibodies were purified from sera obtained 5–8 days after the second biweekly injection of PC-keyhole limpet hemocyanin (KLH) (5 moles PC/100,000 dalton M.W. KLH).

Isoelectric Focusing of Light Chains. Antibodies were completely reduced and alkylated and then isofocused as previously described (Claflin 1976a). Identity of the L chains belonging to the H8 clonotype

Table 1. Alloantisera Used for Typing Mice at *H-2* and *Ly-2,3*

Antiserum Number[a]	Recipient Strain	Donor Strain	Allele Specified
D-2	[B10.A(5R)/Sn × LP.RIII/Sn]F_1	C57BL/10Sn	*H-2D^b*
AS-504	BALB/cBy	HTG/Sn	*H-2D^b*
C-33, D-33	(B10.D2/nSn × A/WySn)F_1	B10.A(5R)/Sn	*H-2K^b*
AS-485	[C3H.SW/Sn × B10.A(2R)/Sn]F_1	C3H/Sn	*H-2D^k*
AS-423	(C57BL/10Sn × C3H-*H-2^0*/Sn)F_1	B10.A(2R)/Sn	*H-2K^k*
D-23	(C57BL/10Sn × LP.RIII/Sn)F_1	B10.A(2R)/Sn	*H-2K^k*
AS-523	C57Br/cdJ	CE/J	*Ly-2^a*
AS-593	(C3H/Sn × BDP/J)F_1	AKR/J	*Ly-3^a*
AS-366	(C3H/Sn × BDP/J)F_1	B10.Y/Sn	*Ly-2^b*

[a] Antisera designated C- or D- were obtained through the Transplantation and Immunology Branch, NIAID, National Institutes of Health

was determined by comparison with H8 L chains. The two recognized variants of H8-clonotype L chains are PC8-A and PC8-B (Claflin 1976a), and the corresponding genotypes are referred to as $PC8^a$ and $PC8^b$. Deliberate mixing of antibodies from both phenotypes in different ratios showed that we could detect both phenotypes when one was present in as little as 1/20 the concentration of the other.

Results

The original publication (Claflin 1976a) presented the PC8 phenotypes of 17 inbred strains. Since that publication, three additional strains—BSVS, NZB/B1NJ, and SWR/J, all Ly-3.2—have been immunized with the T-independent antigen R36A and typed as PC8-B. In crosses C58/J × C57L/J, AKR/J × SWR/J, and C58/J × SWR/J, there was codominant expression of PC8 phenotypes but, as observed previously with (AKR/J × C57L/J)F_1 mice (Claflin 1976a), the PC8-A bands in each case were much more prominent than PC8-B bands.

Additional studies have been conducted on anti-PC antibodies obtained after immunization with the T-dependent antigen PC-KLH (Quintans and Cosenza 1976), which stimulated IgG as well as IgM anti-PC antibody (Claflin and Cubberley, unpublished data). Of 16 inbred strains typed for PC8, all gave the same typing as previously seen when R36A was used as immunogen. However, in the same four F_1 combinations described above, codominant expression of *PC8* alleles was associated with equal representation of parental phenotypes (as determined by band density). Thus, under conditions of stronger stimulation and T-cell helper activity, clonal dominance of PC8-A was overcome.

In no instance have we observed both phenotypes in any inbred strain, even though we deliberately overloaded the gel in order to examine for hidden genes. Thus, the distribution of the PC8-A or PC8-B marker in 20 different inbred mouse strains immunized with R36A is strictly associated with the expression of the T-lymphocyte phenotypic markers Ly-3.1 and Ly-3.2. The availability of RI and congenic mice made it possible to test the Mendelian inheritance of the markers, to establish linkage to the *Ly-2,3* locus, and to estimate the upper limit of the distance between *PC8* and *Ly-2,3*.

Examination of the AKXL RI strains provided initial evidence favoring linkage of *PC8* and *Ly-2,3*. As is shown in Table 2, there was complete concordance of $PC8^a$ with $Ly\text{-}2^a,3^a$ and $PC8^b$ with $Ly\text{-}2^b,3^b$ among the 15 AKXL RI strains studied. RI strains HP/Ei and LT/Sv, typed as $Ly\text{-}2^b,3^b$, were found to be $PC8^b$. *Ly-2,3* (and *PC8*) assorted independently of *H-2* and *Ig-1* in the RI strains, as expected (Itakura *et al.* 1972).

To confirm the linkage, mice congenic to C57BL/6 but carrying the $Ly\text{-}2^a,3^a$ allele of either RF or PL were examined. Both congenics were developed by selecting for the $Ly\text{-}2^a,3^a$ allele; no screening for other neighboring loci, including κ-chain markers, was made. As is shown in Table 2, H8-like antibodies in both congenic strains were found to have L chains of the PC8-A phenotype. The close linkage was reinforced by the finding that even after 22 backcross generations, the *PC8* genotype was still coinherited with *Ly-2,3*. Of the 11 N24 progeny examined, five exhibited the hybrid PC8 phenotype, indicating that selection for heterozygosity at the *Ly-2,3* locus resulted in heterozygosity at the PC8 marker of PL/J.

The question of whether clonal dominance of PC8-A seen in hybrids immunized with R36A is linked to *Ly-2,3* or determined by independent genes can be resolved. All three (C57L × AKXL-28)F_1 mice and four (C57L × AKXL-29)F_1 mice which were typed as heterozygotes for *PC8* contained H8-like L chains that were

Table 2. Segregation of *PC8* and *Ly-2,3* among AKXL RI Lines and Ly Congenic Mice

Mice[a]	Genotypes			
	PC8	*Ly-2,3*	*Ig-1*[b]	*H-2*
RI Line				
AKXL-4	*b*	*b*	*a*	*k*
6	*b*	*b*	*a*	*k*
8	*b*	*b*	*d*	*k*
12	*b*	*b*	*d*	*b*
13	*a*	*a*	*a*	*k*
16	*b*	*b*	*a*	*b*
17	*b*	*b*	*d*	*b*
21	*b*	*b*	*a*	*k*
24	*a*	*a*	*d*	*b*
25	*a*	*a*	*d*	*b*
28	*a*	*a*	*d*	*k*
29	*a*	*a*	*a*	*b*
36	*b*	*b*	*a*	*b*
37	*b*	*b*	*d*	*b*
38	*b*	*b*	*d*	*k*
Congenic mice[c]				
B6.RF-*Ly-2*^{a}*Ly-3*^{a}/Boy(N17F20+)	*a*	*a*	*b*	*b*
B6.PL-*Ly-2*^{a}*Ly-3*^{a}/Cy(N11F10)	*a*	*a*	*b*	*b*
B6 × (B6.PL/B6-*Ly-2*^{a}*Ly-3*^{a}/*Ly-2*^{b}*Ly-3*^{b}N23)[d]	*a*/*b*(5)	NT	*b*	*b*
	b/*b*(6)	NT	*b*	*b*

[a] All mice were immunized with R36A and purified anti-PC antibodies typed for PC8

[b] Typing of RI lines, Taylor *et al.* (1975); typing of congenic strains, this paper

[c] N refers to number of crosses to partner strain before inbreeding began; N-1 = number of backcross generations to partner strain, C57BL/6

[d] These are the progeny in the 23rd backcross generation. The heterozygosity of two of five *a*/*b* mice is assumed since the A phenotype shows dominant expression. The N23 parent was typed as heterozygous at *Ly-2,3*

predominantly PC8-A. Moreover, in the five B6.PL N24 mice typed as *PC8*^{a}/*PC8*^{b}, dominance is seen, despite the fact that the genetic background is C57BL/6. Thus, clonal dominance is intrinsically associated with *PC8*, and does not depend on any other segregating genes, such as *Ig-1*.

Since no recombinants between *PC8* and *Ly-2,3* were observed, we have no estimate of the recombination frequency. However, the upper limit of the recombination frequency (r) can be calculated from the data on the RI strains and the *Ly-2,3* congenics. For the RI strains, the probability of obtaining no recombinants among 17 independently derived lines is $[1 - 4r/(1 + 6r)]^{17}$, where r is the true recombination frequency (Haldane and Waddington 1931). In the breeding of the *Ly-2,3* congenics, there were a total of 41 additional opportunities for recombination, so the probability of no recombination is $(1 - r)^{41}$. Multiplying the two probabilities, and setting the product equal to 0.05 and 0.01, provides the upper 95 and 99 percent confidence limits for r, respectively. The results are 0.026 for the former, and 0.042 for the latter. The actual recombination frequency could be much less than these upper limits. The finding of complete concordance between the V_k-PC8 marker and Ly-3 specificities among 20 inbred strains indicates that this may be the case.

Discussion

This study extends a previous description of an L-chain genetic marker, PC8, to show that it is inherited in a simple Mendelian fashion and that the locus governing its expression is closely linked to the *Ly-2,3* T-lymphocyte surface alloantigen locus on chromosome 6. While the most parsimonious view of the data argues that the PC8 polymorphism reflects variation in the V_k library of different strains, it is possible that we are dealing with a regulatory locus for V_k gene expression which maps on chromosome 6.

We also show that the clonal dominance of PC8-A, observed when F_1 mice are immunized with the T-independent antigen, R36A, is not observed when a T-dependent antigen is used. Whether this is the result of the activity of T helper cells or of the presence of IgG antibody was not determined. Regardless of the mechanism, this finding demonstrates that, under conditions of perhaps more adequate stimulation, the usual gene dosage effects occur.

Other investigators have identified genetic variability among L chains of different inbred mouse strains. Gottlieb and coworkers have described a genetic marker, called I_B, in the *V* region of a minority of mouse L chains (Edelman and Gottlieb 1970, Gottlieb 1974). The degree of heterogeneity of L chains possessing the marker has not yet been determined. Structural studies show that the I_B peptide, as originally identified, is not a single sequence but at least three different sequences. More recently, Gibson (1976, 1977), using the technique of IEF to separate pooled normal mouse chains, has observed variation in the spectrum of L-chain bands in the same inbred strains that are I_B-positive. The variants are limited to from three to four bands in a total of about 50 and is, therefore, most likely a V-region, rather than a C-region marker. With both I_B and the IEF variants, expression is closely associated with the *Ly-2,3* locus.

To evaluate the significance of PC8, as well as I_B and the IEF variants, it is necessary to consider the limitations of the systems described to date. In the first place, structural studies which would prove whether the products of one or multiple genes are being examined are lacking. Analysis of the existing data would argue for the latter. The Gottlieb I_B marker is clearly located around the first half-cystine (cys23). But multiple sequences exist in I_B, and because this is a region of low variability in mouse κ chains (Kabat *et al.* 1976), one might expect that I_B would be found in more than one V_κ subgroup and, determined in turn, by more than one gene (assuming a minimum of one gene for each subgroup). Specifically, sequences from the hexapeptides I_C and I_D are each found in a minimum of two V_κ subgroups (Kabat *et al.* 1976, Potter 1977). Gibson's IEF variants are multiple and have quite different pIs. This approach is undoubtedly revealing different V_κ subgroups. PC8 is a single V_κ subgroup whose spectrotypic pattern in the reference strains is the reverse of that seen in the IEF variants that Gibson describes for that pI range. Moreover, as discussed previously, I_B cannot represent the PC8 marker without some compensatory charge changes in the reverse direction occurring elsewhere in the *V* region. PC8 variants can easily be accounted for by a neutral amino acid to histidine or tyrosine to neutral amino acid interchange. Thus, it seems reasonable to conclude that the different approaches are revealing different V_κ subgroups, which are clustered together much like mouse V_H genes (Riblet *et al.* 1975, Weigert and Potter 1977).

A second consideration is the potential problem of identifying alleles at a *V*-region locus. The alternative bands associated with the A and B phenotypes could be

considered alleles. If this were so, one would expect to find only a few amino acid differences between them. However, the definition of allelism is blurred when one deals with tandemly arranged duplicated genes. For arrangements of this type there is strong evidence for unequal homologous crossovers with accompanying deletions and duplications. For instance, AKR/J hemoglobin possesses two different β chains, β^{dmaj} and β^{dmin}, while C57L/J possesses a third type, βs. Each of these β chains differs at multiple amino acid residues from one another (Gilman 1972, Popp 1973, Popp and Baileff 1973). Genes coding for the β chains map to a single locus, *Hbb* on chromosome 7, but in crosses between Hbb^{d} and Hbb^{s}, Hbb^{dmaj}, and Hbb^{dmin} behave as a gene doublet allelic to Hbb^{s}.

An accompanying complication is that of regulation of expression of *V*-gene products. The absence of a V-region phenotype in a strain, such as when an idiotype is recognized in one strain but not others, may be interpreted as allelic variation, as regulation of expression, or as an example of gene deletion. Such a situation has been encountered for the T15-M511 idiotype in BALB/c, C57BL/6, and A mice. Serum of BALB/c, but not C57BL/6 or A, mice contains antibody bearing the T15-M511 idiotype (A/He anti-T15), but BALB/c and C57BL/6, not A, possess B cells capable of expressing the T15-M511 idiotype (Cancro *et al.* 1978). On the one hand, an IEF polymorphism in the normal pool of L chains occurring within a narrow PI range may represent alleles at a single locus (though not necessarily so), but the existence of an L-chain band (or I_B) in one strain and its absence in a corresponding, but nonidentical, position in another strain cannot be so easily explained. Is this an example of deletion or of differential regulation of the same gene or its product?

The data presented in this paper clearly define a locus linked to *Ly-2,3* which controls the PC8 marker. However, from the data presently available, it is not possible to establish the nature of the genetic locus controlling this trait. This point has been emphasized by Gottlieb and Durda (1977). Four major hypotheses exist: (1) *PC8* is a polymorphic structural locus coding for the L chains of antibodies in mice idiotypically identical to H8. (2) *PC8* is a regulatory gene that controls the expression of H8 L chains. This hypothesis implies that both H8 L-chain genes are present in the genome but only one is expressed. (3) A single L-chain gene exists but an epigenetic (*e.g.*, enzymatic) event modifies the L chain in one strain but not the other. (4) The *Ly-2,3* locus codes for V_κ. This last hypothesis is ruled out by the fact that the Ly-3 surface antigen is a glycoprotein which does not resemble immunoglobulin κ chains (Durda and Gottlieb 1976). The third possibility is also unlikely. A candidate for modification would be sialic acid residues or, possibly, amino acids. But neither serum from mice bearing the alternate phenotype (Claflin 1976a) nor neuraminidase (Claflin, unpublished data) has any effect on the trait. It is also difficult to see how post-translational alteration of amino acid side chains would lead to such a minor effect. Moreover, one would anticipate dominance of one phenotype in heterozygotes and this was not observed under conditions of adequate stimulation, *i.e.*, immunization with PC-KLH.

To eliminate or choose one of the remaining two hypotheses is more difficult. The simplest hypothesis is that the PC8 marker reflects structural gene variation in V_κ. One might expect to find polymorphism in V_κ in view of the extensive polymorphism found in V_H. The fact that no polymorphism has been found in C_κ eliminates for now the possibility of using linkage to C_κ as one criterion for judging the nature of the PC8 marker. It is difficult to envision a series of regulatory loci, one for each V_κ gene or, alternatively, a mechanism whereby a single locus would regulate one of a set of V_κ genes. Finally, the fact that no deviation from allelic behavior has been seen in

any of 20 inbred strains, 17 RI lines, and two congenic strains supports a structural gene concept. One could argue that a locus adjacent to *Ly-2,3* (or in fact, *Ly-2,3* itself) operates via a different cell to direct expression (positively or negatively) by a set of B cells bearing certain L chains. The existence of latent allotypes in mice (Bosma and Bosma 1974) and rabbits (Strosberg *et al.* 1974, Mudgett *et al.* 1975) has been reported, and it is possible that our assay was not sufficiently sensitive to detect the latent marker. One might also point to the differential pattern observed in R36A versus PC-KLH-immunized heterozygotes as demonstrating regulation. However, we have no evidence that the dominant expression of PC8-A is related to *Ly-2,3*. We may have observed a simple clonal dominance phenomenon resulting from the fact that PC8 affects antigen binding properties and/or from the stronger influence on the B-cell pool obtained with using a T-dependent antigen.

In conclusion, one may ask the purpose of being especially critical of a phenomenon which classically would be interpreted as strong evidence for a structural gene locus. In addition to the probable existence of anomalous allotypes and to idiotype regulation in BALB/c, there is preliminary evidence, from hybridization of κ-chain cDNA to DNA from mouse-hamster and mouse-human hybrid cell lines, that chromosome 6 may not contain κ-chain sequences (Swan and Leinwand, personal communication). If these data are verified in subsequent experiments, the idea of a regulatory locus must be considered seriously. At present, however, the weight of evidence favors a structural gene locus.

The very close linkage of the kappa locus to the *Ly-2,3* T-lymphocyte locus is provocative. It seems unlikely that these loci would be so closely linked solely by coincidence. Close linkage could result from either a functional or an evolutionary relationship. Ly-2,3 antigens are found on the surface of T-effector cells, but not helper T cells (Jandinski *et al.* 1976). Immunoglobulin is certainly on B cells and at least the variable region portion may also be present on T cells (Black *et al.* 1976, Binz and Wigzell 1975). One model would suggest that T and B cells share variable regions and that Ly-2,3 antigens are composed of constant and variable regions that could function as a receptor. Anti-Ly-2,3 antisera would detect determinants located in the constant region. As discussed previously, Gottlieb could find no evidence that Ly-2,3 antigens from appropriate strains contain the I_B marker, as would be expected if *V*-region sequences were shared (Durda and Gottlieb 1976). There might be an evolutionary relationship between *Ly-2,3* and kappa light chains, such that both evolved by tandem duplication from a common ancestral gene. Given sufficient time, completely distinct functions might evolve, while the close linkage persisted. When sequence data are available for Ly-2,3 antigens, it may be possible to evaluate these ideas. We are currently examining the structure of the PC8 marker and looking for recombinants between *PC8* and *Ly-2,3* in wild mice which may provide information on these choices.

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References

Binz, H. and Wigzell, H.: Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants. I. Demonstration of similar or identical idiotypes on IgG molecules and T-cell receptors with specificity for the same alloantigen. *J. Exp. Med. 142*:197–211, 1975

Black, S.J., Hammerling, G., Berek, C., Rajewsky, K., and Eichmann, K.: Idiotypic analysis of lymphocytes *in vitro*. I. Specificity and heterogeneity of T and B lymphocytes reactive with anti-idiotypic antibodies. *J. Exp. Med. 143*:846–860, 1976

Bosma, M. and Bosma, G.: Congenic mouse strains: the expression of a hidden immunoglobulin allotype in a congenic partner strain of BALB/c mice. *J. Exp. Med. 139*:512–527, 1974

Boyse, E.A., Itakura, K., Stockert, E., Iritani, C.A., and Miura, M.: Ly-C: a third locus specifying allo-antigens expressed only on thymocytes and lymphocytes. *Transplantation 11*:351–353, 1971

Cancro, M., Sigal, N., and Klinman, N.: Differential expression of an equivalent clonotype among BALB/c and C57BL/6 mice. *J. Exp. Med. 147*:1–12, 1978

Cantor, H. and Boyse, E.A.: Functional subclasses of T lymphocytes bearing different Ly antigens. *J. Exp. Med. 141*:1376–1389, 1975

Claflin, J.L.: Genetic marker in the variable region of kappa chains of mouse anti-phosphorylcholine antibodies. *Eur. J. Immunol. 6*:666–668, 1976a

Claflin, J.L.: Uniformity in the clonal repertoire for the immune response to phosphorylcholine in mice. *Eur. J. Immunol. 6*:669–674, 1976b

Claflin, J.L. and Davie, J.M.: Clonal nature of the immune response to phosphorylcholine. III. Species-specific binding characteristics of rodent anti-phosphorylcholine antibodies. *J. Immunol. 113*:1678–1684, 1974

Durda, P.J. and Gottlieb, P.D.: The Ly-3 antigens on mouse thymocytes: immune precipitation and molecular weight characterization. *J. Exp. Med. 144*:476–493, 1976

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

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

Gibson, D.: Genetic polymorphism of mouse immunoglobulin light chains revealed by isoelectric focusing. *Immunogenetics 4*:420, 1977

Gilman, J.G.: Hemoglobin beta-chain structure: evolutionary and functional implications. *Science 178*:873–874, 1972

Gottlieb, P.D.: Genetic correlation of a mouse light chain variable region marker with a thymocyte surface antigen. *J. Exp. Med. 140*:1432–1437, 1974

Gottlieb, P.D. and Durda, P. J.: The I_B-peptide marker and the Ly-3 surface alloantigen: structural studies of a V-region polymorphism and a T-cell marker determined by linked genes. *Cold Spring Harbor Symp. Quant. Biol. 41*:805–815, 1977

Haldane, J.B.S. and Waddington, C.H.: Inbreeding and linkage. *Genetics 16*:357–374, 1931

Itakura, K., Hutton, J.J., Boyse, E.A., and Old, L.J.: Genetic linkage relationships of loci specifying differentiation alloantigens in the mouse. *Transplantation 13*:239–243, 1972

Jandinski, J., Cantor, H., Tadakuma, T., Peavy, D.L., and Pierce, C.W.: Separation of helper T cells from suppressor T cells expressing different Ly components. I. Polyclonal activation: suppressor and helper activities are inherent properties of distinct T-cell subclasses. *J. Exp. Med. 143*:1382–1390, 1976

Kabat, E.A., Wu, T.T., and Bilofsky, H.: *Variable Regions of Immunoglobulin Chains*. Bolt, Beranek, and Newman, Cambridge, 1976

Mudgett, M., Fraser, B.A., and Kindt, T.J.: Nonallelic behavior of rabbit variable region allotypes. *J. Exp. Med. 141*:1448–1452, 1975

Popp, R.A.: Sequence of amino acids in the β chain of single hemoglobins C57BL, SWR and NB mice. *Biochem. Biophys. Acta 303*:61–67, 1973

Popp, R.A. and Baileff, E.G.: Sequence of amino acids in the major and minor β chains of the diffuse hemoglobin from BALB/c mice. *Biochem. Biophys. Acta 303*:61–67, 1973

Potter, M.: Antigen binding myeloma proteins of mice. *Adv. Immunol.*, in press, 1977

Potter, M. and Lieberman, R.: Genetics of immunoglobulins in the mouse. *Adv. Immunol. 7*:145, 1967

Quintans, J. and Cosenza, H.: Antibody response to phosphorylcholine *in vitro*. II. Analysis of T-dependent and T-dependent responses. *Eur. J. Immunol. 6*:399–405, 1976

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

Snell, G.D., Démant, P., and Cherry, M.: Hemagglutination and cytotoxic studies of *H-2*. I. H-2.1 and related specificities in the EK crossover regions. *Transplantation 11*: 210–237, 1971

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

Taylor, B.A. and Meier, H.: Mapping the adrenal lipid deletion gene of the AKR/J mouse strain. *Genet. Res. 26*:307–312, 1976

Taylor, B.A., Bailey, D.W., Cherry, M., Riblet, R., and Weigert, M.: Genes for immunoglobulin heavy chains and serum albumin are linked in the mouse. *Nature 256*:644–646, 1975
Weigert, M. and Potter, M.: Antibody variable-region genetics. *Immunogenetics 4*:401–425, 1977

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