ON A REGULATORY GENE CONTROLLING THE EXPRESSION OF THE MURINE λ_1 LIGHT CHAIN*

BY WILLIAM GECKELER, JEROME FAVERSHAM, AND MELVIN COHN

From the Developmental Biology Laboratory, The Salk Institute for Biological Studies, San Diego, California 92112

The λ_1[1] light chain is encoded by a single germ-line v_{λ_1} and c_{λ_1} gene. Upon committment, the v_{λ_1} gene is translocated in *cis* to a transcription unit which includes the c_{λ_1} gene separated from v_{λ_1} by a nontranslated stretch of some 1,250 nucleotides (intron) (1). This unit is then transcribed as the high molecular weight nuclear RNA (HnRHA) which must be processed by excision of the intron to yield the polysome-bound mRNA in which the coding of v_{λ_1} and c_{λ_1} must be contiguous (2, 3).

The v_{λ_1} germ-line gene complemented with a set of closely-related heavy chain variable region (v_H) germ-line genes (referred to as $v_{H\lambda_1}^{\alpha(1,3)}$) encodes the anti-$\alpha$(1,3)dextran specificity of high responder strains of mice. The role of the $v_{H\lambda_1}^{\alpha(1,3)}$ genes in the anti-α(1,3) response has been extensively studied (4). Here we deal with the genetics of expression of the λ_1 light chain, both in normal immunoglobulin and in the antibody to α(1,3)dextran. This is the first example we have of a regulatory gene linked to the light chain locus which controls specific dominant responsiveness.

Materials and Methods

Most of the Materials and Methods have been described in detail elsewhere (5).

Quantitation of the Light Chain Class Associated with Antibody to the α(1,3) and α(1,6) Glucosyl Linkages of B1355. A two-stage radioimmunoassay (RIA) was used to quantitate both the

* Supported by grant R01 AI05875, awarded by the Allergy and Infections Disease Branch of the National Institutes of Health.

[1] *Abbreviations used in this paper:* ASC, antigen-sensitive, bone-marrow derived lymphocytes; BSA, bovine serum albumin; c_{λ_1}, constant region of the murine lambda-one light chain, or the structural gene encoding it; FITC, fluorescein isothiocyanate; HnRNA, high molecular weight nuclear RNA; Ig, immunoglobulin; Ig-1^a, the *a* allele of Ig-1, which identifies a heavy chain gene complex containing $v_{H\lambda_1}^{\alpha(1,3)}$ genes ($v_{H\lambda_1}^{\alpha(1,3)^+}$); Ig-1^b, the *b* allele of Ig-1, which identifies a heavy chain gene complex lacking $v_{H\lambda_1}^{\alpha(1,3)}$ genes ($v_{H\lambda_1}^{\alpha(1,3)^-}$); κ, the murine kappa light chain, defined by the C-terminal trypic peptide ALA ASP CYS; λ_1, the murine lambda-one light chain, defined by the C-terminal trypic peptide ALA ASP CYS SER; r_{λ_1} locus, a gene locus regulating λ_1 light chain expression; $r_{\lambda_1}+$, an allele of the λ_1 locus present in the BALB/c strain; r_{λ_1}lo, an allele of the λ_1 locus present in the SJL strain; λ_2, the murine lambda-two light chain, defined by the C-terminal trypic peptide SER LEU SER PRO ALA GLU CYS LEU; NRS, normal rabbit serum; PBSAE, phosphate-buffered saline with 10^{-3} M sodium azide and 10^{-3} M (ethylenedinitrilo)-tetraacetic acid; RIA, radioimmunoassay; v_H, variable region of the heavy chain or the structural gene encoding it; $v_{H\lambda_1}^{\alpha(1,3)}$, one of several germ-line heavy chain variable regions, or the gene encoding it, which complements with v_{λ_1} to produce a combining site with specificity for the α(1,3) glucosyl linkage; v_κ, variable region of the kappa light chain or the structural gene encoding it; v_L, variable region of the light chain or the structural gene encoding it; v_{λ_1}, variable region of the lambda-one light chain or the structural gene encoding it; v_{λ_2}, variable region of the lambda-two light chain or the structural gene encoding it; $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$, the possession of both a v_{λ_1} structural gene and also a $v_{H\lambda_1}^{\alpha(1,3)}$ set, such that the association of the products of the v_{λ_1} structural and any one of the $v_{H\lambda_1}^{\alpha(1,3)}$ genes produces a combining site with specificity for the α(1,3) glucosyl linkage.

J. EXP. MED. © The Rockefeller University Press · 0022-1007/78/1101-1122$1.00
Volume 148 November 1978 1122–1136

fraction of a B1355 antibody response with specificity for each linkage and also the κ:λ_1 ratio of the α(1,3) and α(1,6) antibody response.

B1355, containing α(1,3) and α(1,6) glucosyl linkages, or B512, which contains α(1,6) linkages are the gift of Dr. Allene Jeanes, Department of Agriculture, Peoria, Ill. Each is dissolved to a final concentration of 100 μg/ml in phosphate-buffered saline with 10^{-3} M sodium azide and 10^{-3} M (ethylenedinitrilo)-tetraacetic acid (PBSAE). 1 ml of either dextran solution is added to polystyrene tubes (Falcon 2052, Falcon Plastics, Oxnard, Calif.) and incubated 4 h at room temperature to allow adsorption of dextran into the tubes. The tubes are washed three times with PBSAE and remaining adsorption sites are blocked by a ½ h incubation with 2 ml of 1% (wt/vol) bovine serum albumin (BSA) in PBSAE. The anti-B1355 titer of immune test sera is estimated by hemagglutination assay. In the first stage of the RIA, an amount of test antiserum diluted to 1.1 ml with 1% (wt/vol) BSA in PBSAE is added to two tubes with absorbed B1355 (α(1,3) + α(1,6)) and to two tubes with adsorbed B512 (α(1,6) only) such that neither the α(1,3) nor the α(1,6) binding sites are saturated by the anti-dextran antibody (generally $<$10 μg). For each test sera there are now two tubes with bound B1355 dextran/anti-dextran antibody complexes and two tubes with bound B512 dextran/anti-dextran complexes.

In the second stage of the RIA, the amount of κ- and λ_1-bearing immunoglobulins in these complexes is determined. ^{125}I-labeled anti-κ, diluted to 1.2 ml in 1% (wt/vol) BSA and 0.1% normal rabbit serum (NRS) in PBSAE, is added to one of the two tubes containing either dextran/anti-dextran antibody complex. The other tube in each set is likewise incubated with ^{125}I-labeled anti-λ_1 antibody. The amount of added anti-light chain is in excess of the amount which is capable of binding to the dextran/anti-dextran complex. The second stage of the RIA is incubated for 24 h. The tubes are washed three times with PBSAE, dried, and counted for ^{125}I content. The amount of ^{125}I-labeled anti-κ or anti-λ_1 antibody which has bound to each dextran/anti-dextran complex is a function of the amount of anti-dextran antibody in the complex. This is quantitated by reference to a standard curve. The standard curve for bound λ_1 anti-dextran/^{125}I-labeled anti-λ_1 is constructed by binding known amounts of purified J558, a λ_1 plasmacytoma antibody with α(1,3)specificity, to B1355 dextran in the first stage, and subsequently measuring the amount of ^{125}I-labeled anti-λ_1 antibody bound in the second stage.

A standard curve is likewise constructed for bound κ-anti-dextran/^{125}I-labeled anti-κ by binding known amounts of purified W3129, a κ-plasmacytoma antibody with α(1,6)specificity, to B512 dextran in the first stage, and subsequently measuring the amount of ^{125}I-labeled anti-κ antibody bound in the second stage. The amount of antibody with α(1,3)specificity is calculated by subtracting the amount of antibody bound to α(1,6)determinants (B512) from the amount bound to α(1,3) and α(1,6)determinants (B1355).

Preparation of Anti-Idiotypic Antibody. An anti-idiotypic antibody specific for the combining site of anti-α(1,3)dextran antibodies encoded by $v_{\lambda_1}v_{H\lambda_1}^{\alpha 1,3}$ was prepared by immunizing rabbits with MOPC 104E (λ_1 IgM anti-α(1,3)dextran). Most of the anti-λ_1 antibody was removed by affinity chromatography of the anti-serum on Y 5431 (λ_1 Bence-Jones) coupled to Sepharose 4B. The effluent was chromatographed on a Sepharose 4B column to which J558 (λ_1 IgA anti-α(1,3)dextran), which idiotypically cross-reacts with MOPC 104E, was coupled. The acid eluate of this column was neutralized and passed over a Y5606 (λ_1IgG$_3$) Sepharose 4B column to remove residual anti-λ_1 activity, and the anti-idiotypic antibody was obtained in the effluent. The binding of the purified anti-idiotypic antibody can be specifically inhibited by α(1,3)containing dextrans.

Results

The Amount of λ_1-Light Chain Associated with Normal Serum Immunoglobulin is different in Various Mouse Strains. Among most inbred mouse strains there is no more than a fourfold variation in λ_1 levels (Table I). Two inbred strains, however, SJL and BSVS, have very low levels of λ_1. The reduction in λ_1 serum levels is specific for the λ_1 light chain class since inbred strains with low or high levels of λ_1 have comparable levels of the κ-light chain in normal serum.

To demonstrate that strains with very low measured levels of λ_1 actually possess a finite amount of λ_1Ig, portions of SJL normal serum were preincubated with anti-λ_1

TABLE I

The Amount of λ_1 and κ-Immunoglobulin in Normal Serum of Various Mouse Strains

Mouse strain	λ_1Ig	κ-Ig	λ_1:κ
	μg/ml serum	*μg/ml serum*	%
BALB/c	68.0	4,400	1.5*
BALB/c (nu/nu)	66.0	—	—
SJL	2.5	7,000	0.035
SJA_9	0.45	—	—
BSVS	1.0	3,500	0.028
BRVR	43.0	—	—
(SJLxBSVS)F_1	1.8	—	—
B10.D2	88.0	—	—
TL-A	84.0	—	—
LP	78.0	—	—
129	50.0	—	—
B10.BR	48.0	—	—
SL	40.0	—	—
A/He	39.0	—	—
C57L	39.0	—	—
CWB	39.0	—	—
W/S	39.0	—	—
C3H.Q15	28.0	—	—
C57BL/6	24.0	3,000	0.80
RIII	24.0	—	—

Determinations were performed by RIA of serum pooled from at least 10 individual mice of both sexes. SE ≤0.03 for all determinations. —, not done.

* Our data show a lower λ_1:κ-ratio than that reported in a recent study (14) using radiochemical determination of carboxyl-terminal peptides. While this difference does not affect our interpretations of the findings, the reason for the discrepancy remains to be clarified.

coupled to Sepharose. Such preincubation abrogates by 99% the ability of the serum to compete in the RIA for λ_1 (data not shown). We conclude that the low values of λ_1Ig in SJL and BSVS are real and that the λ_1 level in BALB/c normal serum is 30-fold higher than in SJL. When a comparison is made of λ_1Ig levels relative to κ-Ig levels (the λ_1:κ-ratio) the λ_1Ig level is 50-fold higher in BALB/c than in SJL.

The Expression of λ_1 is Controlled by a Single Genetic Locus. To analyze the genetics of the control of λ_1 expression, (BALB/c × SJL)F_1, F_2, and F_3 progeny were examined. BALB/c has high levels of λ_1 and SJL has low levels of λ_1. These inbred strains possess different markers at the two gene complexes which are known to control specific dominant immune responsiveness, the major histocompatibility complex and the heavy chain complex. BALB/c has the $H\text{-}2^d$ haplotype and the heavy chain complex identified by the $Ig\text{-}1^a$ allotype. SJL has the $H\text{-}2^s$ haplotype and the $Ig\text{-}1^b$ allotype (6).

(BALB/c × SJL)F_1 progeny are phenotypically λ_1 intermediates, i.e., their average λ_1 level is one-half that of the λ_1 high phenotype (Results).

(BALB/c × SJL)F_2 progeny were typed for their H-2 haplotypes and heavy chain allotypes. The F_2 progeny homozygous for all combinations of H-2 haplotype and heavy chain allogroup were tested for λ_1 serum levels. The results are shown in Fig.

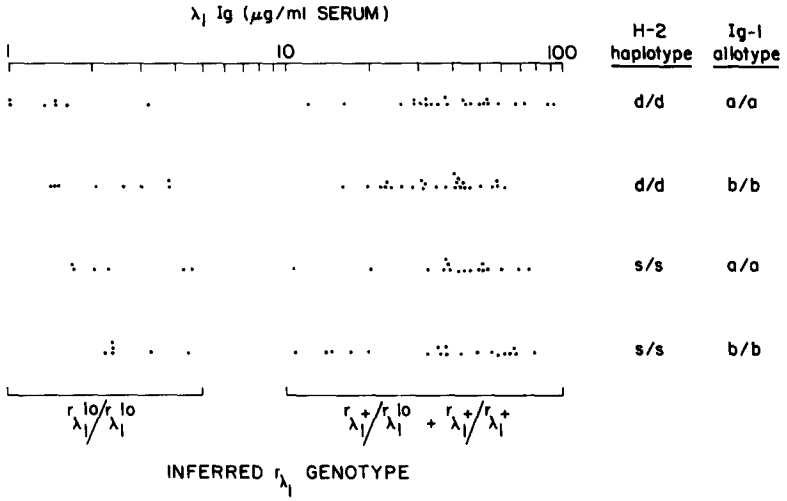

FIG. 1. The amount of λ_1Ig in the serum of (BALB/c × SJL)F_2 progeny homozygous for H-2 haplotype and heavy chain allogroup. (BALB/ c × SJL)F_2 progeny homozygous for H-2 haplotype and heavy chain allogroup were tested for serum levels of λ_1Ig by RIA. Individuals with less than 5 μg/ml λ_1Ig are phenotypically λ_1 low and are postulated to possess the r_{λ_1}lo/r_{λ_1}lo genotype. Individuals with greater than 10 μg/ml λ_1Ig are phenotypically λ_1 intermediate or λ_1 high, and are postulated to possess the $r_{\lambda_1}+/r_{\lambda_1}$lo or $r_{\lambda_1}+/r_{\lambda_1}+$ genotype, respectively.

TABLE II

Segregation in the (BALB/c × SJL)F_2 Generation of the r_{λ_1} Locus as a Single Genetic Unit, Unlinked to the Major Histocompatibility Complex (H-2) or to the Heavy Chain Allogroup

Observed λ_1 phenotype	Postulated genotype	H-$2^{d/d}$Ig$^{a/a}$	H-$2^{d/d}$Ig$^{b/b}$	H-$2^{a/a}$Ig$^{a/a}$	H-$2^{a/a}$Ig$^{b/b}$
Low	r_{λ_1}lo/r_{λ_1}lo	7	8	6	6
Intermediate to high	r_{λ_1}lo/r_{λ_1}+ + $r_{\lambda_1}+/r_{\lambda_1}+$	24	26	17	19
Number of H-2 and heavy chain allogroup homozygotes		31	34	23	25

An SJL × BALB/c mating comprised the parental generation for the genetic segregation study. SJL mice are homozygous r_{λ_1}lo, H-2^a, Ig-1^b. BALB/c mice are homozygous $r_{\lambda_1}+$, H-2^d, Ig-1^a. 512 F_2 progeny were tested for H-2 haplotype and heavy chain allotype. Individuals homozygous for each combination of H-2 and allotype were then tested for the level of λ_1Ig in normal serum. Of 512 mice 1/16 (32) are expected to be homozygous at both alleles for any combination of H-2 and heavy chain allogroup. Within each category 1/4 are expected to be r_{λ_1}lo/r_{λ_1}lo and 3/4 are expected to be r_{λ_1}lo/r_{λ_1}+ plus $r_{\lambda_1}+/r_{\lambda_1}+$.

1 and summarized in Table II. Within each group of H-2 and allogroup homozygotes both λ_1 intermediate to high phenotypes and also λ_1 low phenotypes were recovered. The ratio of λ_1 intermediate to high:λ_1 low penotypes within each group was 3:1.

The simplest interpretation of the segregation ratios of high and low λ_1 phenotypes is that two alleles, $r_{\lambda_1}+$ and r_{λ_1}lo, at a single genetic locus, r_{λ_1}, control the level of λ_1 light chain. Thus, BALB/c ($r_{\lambda_1}+/r_{\lambda_1}+$) has the λ_1 high phenotype and SJL (r_{λ_1}lo/r_{λ_1}lo) has the λ_1 low phenotype. In the F_2, the genotypes $r_{\lambda_1}+/r_{\lambda_1}+$, $r_{\lambda_1}+/r_{\lambda_1}$lo, and r_{λ_1}lo/r_{λ_1}lo are expected to distribute in a 1:2:1 ratio. It is difficult to distinguish consistently whether an individual is λ_1 high or λ_1 intermediate, since the phenotype within each genotype group is obscured by individual variation. On the other hand, it is always possible to distinguish λ_1 low from λ_1 high or λ_1 intermediate

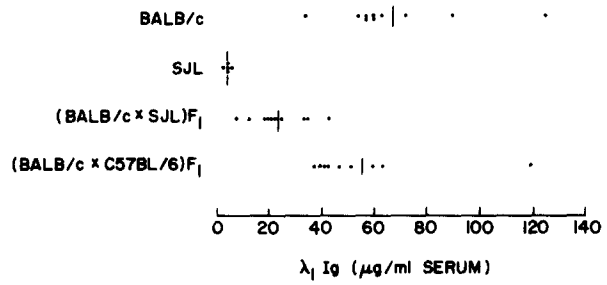

FIG. 2. λ_1Ig levels of(BALB/c × SJL)F_1 individuals measured by RIA. Determinations of parental λ_1Ig levels are shown for comparison. λ_1Ig levels of (BALB/c × C57BL/6)F_1 individuals were also determined. C57BL/6, like, SJL, possesses the IG-1^b heavy chain allotype. Unlike SJL, however, C57BL/6 exhibits the λ_1 high phenotype. A vertical bar represents the mean λ_1Ig level for each group.

because the difference is very great. Since the observed 3:1 ratio is found in all H-2 haplotype and heavy chain allogroup homozygote classes in the F_2, the r_{λ_1} locus must be unlinked to either the H-2 or the heavy chain gene complexes. Further, the r_{λ_1} locus is not sex-linked.

F_2 mice homozygous for every combination of H-2 haplotype and heavy chain allogroup, and which were postulated because of their phenotypes to be homozygous for $r_{\lambda_1}+$ or r_{λ_1}lo, were mated. In all cases, mating pairs possessing the highest λ_1 levels (presumed $r_{\lambda_1}+/r_{\lambda_1}+$) yielded F_3 progeny which were all λ_1 high, while mating pairs chosen as r_{λ_1}lo/r_{λ_1}lo yielded F_3 progeny all of which were phenotypically λ_1 low. These results confirm that λ_1 expression breeds true and its controlled by a single genetic locus.

The λ_1 Locus is Linked to (or Identical with) the Structural Gene Locus for λ_1. In (BALB/c × SJL)F_1 progeny the $r_{\lambda_1}+$ and r_{λ_1}lo alleles show a gene dosage effect. The λ_1 level of (BALB/c × SJL)F_1 progeny are shown in Fig. 2. For comparison, λ_1 levels of (BALB/c × C57BL/6)F_1 progeny are also shown. The C57BL/6 strain, like SJL, has the Ig-1^b heavy chain allogroup, but unlike SJL expresses a high level of λ_1. The results show that the average level of λ_1 in (BALB/c × SJL)F_1 normal immunoglobulin is one-half the level found in (BALB/c × C57BL/6)F_1 progeny.

The finding that $r_{\lambda_1}+/r_{\lambda_1}$lo heterozygotes have one-half the level of λ_1Ig compared to $r_{\lambda_1}+/r_{\lambda_1}+$ homozygotes implies either that the r_{λ_1} locus is identical to the structural gene locus for λ_1 or that the r_{λ_1} locus is regulatory and expressed in *cis* with the λ_1 structural gene and therefore is closely linked to it. This finding will be dealt with in the Discussion.

The λ_1 Locus Affects the Antibody Response to the $\alpha(1,3)$Glucosyl Linkage which is Encoded by the $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$ Genes. To analyze the mechanism by which the r_{λ_1} locus regulates λ_1 light chain expression, mice homozygous for the Ig-1^a allotype and either the $r_{\lambda_1}+$ or r_{λ_1}lo allele were immunized with B1355. It is known that $r_{\lambda_1}+/r_{\lambda_1}+$ mice possessing the $v_{H\lambda_1}^{\alpha(1,3)^+}$ alleles respond predominantly to the $\alpha(1,3)$ glucosyl linkages of B3155 by producing an antibody encoded exclusively by the complementing genes $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)^+}$ (7). The $v_{H\lambda_1}^{\alpha(1,3)^+}$ genes are present in the heavy chain gene complex identified by the Ig-1^a allotypic marker. The response is uniformly of high magnitude in individuals with the $r_{\lambda_1}+$, Ig-1^a genotype. BALB/c, one of the parents used in the genetic analysis of the r_{λ_1} locus, possesses this genotype. SJL, the other parent used in the genetic analysis of the r_{λ_1} locus, is $v_{H\lambda_1}^{\alpha(1,3)^-}$ (linked to Ig-1^b) and would be a low responder to B1355, even if it were $r_{\lambda_1}+/r_{\lambda_1}+$.

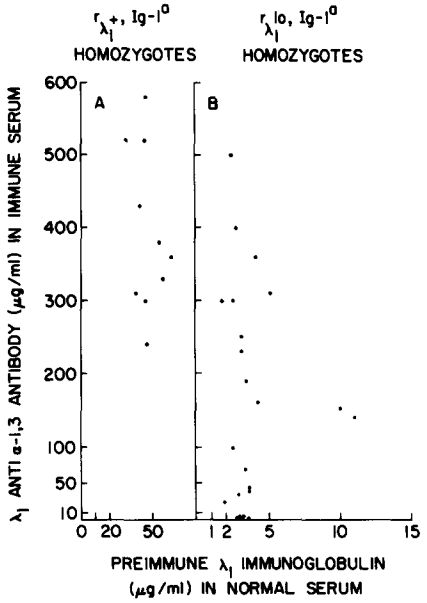

FIG. 3. Magnitude of the λ_1 anti-α(1,3)primary response in r_{λ_1}+, Ig-1^a and r_{λ_1}lo, Ig-1^a homozygote (BALB/c × SJL)F_3 mice measured by a two-stage RIA. (BALB/c × SJL)F_3 mice were allotyped and tested for the amount of λ_1Ig in their serum. Those classified as homozygous for r_{λ_1}+, Ig-1^a or r_{λ_1}lo, Ig-1^a were then immunized i.p. with 100 μg B1355 in PBS and bled 7 days later. Panel A represents the analysis of r_{λ_1}+, Ig-1^a homozygotes and panel B represents the analysis of r_{λ_1}lo, Ig-1^a homozygotes.

(BALB/c × SJL)F_3 mice, homozygous for the Ig-1^a heavy chain allotype and either the r_{λ_1}+ or the r_{λ_1}lo allele, were immunized with B1355. Their λ_1-antibody response to the α(1,3) glycosyl linkage is shown in Fig. 3. r_{λ_1}+, Ig-1^a homozygotes give a uniformly high response like the BALB/c parental strain. Its magnitude varies no more than 2.5-fold among individuals. On the other hand, r_{λ_1}lo, Ig-1^a homozygotes exhibit great individual variation in the magnitude of their λ_1 anti-α(1,3) response. From Table III it can be seen that the anti-α(1,3) response of these r_{λ_1}lo homozygotes can be classified into three groups, high, intermediate, and low. The antibody of high responders is predominantly in the λ_1 light chain class. Intermediate responders fall into three classes; those whose response is in the λ_1 light chain class alone, those whose response is in both λ_1 and κ-light chain class, and those whose response is only in the κ-light chain class. The antibody of low responders is predominantly in the κ-class. SJA_9 mice, which are congenic with SJL except for the Ig-1^a allotype, and thus possess r_{λ_1}lo and Ig-1^a on a uniform genetic background, show the same variability in responsiveness to B1355 (R. Riblet, personal communication). Thus, the wide fluctuation in the magnitude of the α(1,3) dextran response seen in (BALB/c × SJL)F_3 mice homozygous for r_{λ_1}lo and Ig-1^a is unlikely due to genetic loci other than λ_1 which are still segregating. Rather it is due to the r_{λ_1}lo allele itself.

The r_{λ_1}lo allele generates the wide fluctuation in the magnitude of the α(1,3) dextran response among r_{λ_1}lo, Ig-1^a homozygotes by a process which acts randomly in each animal. Thus, if one intermates F_3 r_{λ_1}lo, Ig-1^a homozygotes which were the highest responders to B1355 or the lowest responders to B3155, one finds that the progeny of either mating exhibit the same pattern of wide response variability (data not shown).

TABLE III

Light Chain Class and Determinant Specificity of the Primary Antibody Response to B1355 in (BALB/c × SJL)F_3 Mice Homozygous for $r_{\lambda_1}lo$ and Ig-1^a

Magnitude of the response to B1355 Hemagglutination titer (log_2)		Magnitude of specific responses				Predominant light chain class of antibody
		$\lambda_1$anti-α(1,3)	κ-anti-α(1,3)	$\lambda_1$anti-α(1,6)	κ-anti-α(1,6)	
			μg/ml			
High	>11.5	552	23	<5	<5	λ_1
	>11.5	475	52	<5	<5	λ_1
	>11.5	328	12	<4	<4	λ_1
	10.5	160	19	<4	7	λ_1
Intermediate	8.5	60	6.4	<5	<5	λ_1
	7.5	31	1.8	<5	<5	λ_1
	8.5	44	20	<3	<3	$\lambda_1 + \kappa$
	8.5	<1.25	52.5	<1.25	<1.25	κ
	8.5	<2.5	65	<2.5	<2.5	κ
Low	6.0	<2	32	<2	<2	κ
	5.0	<3	36	<3	<3	κ

(BALB/c × SJL)F_3 mice homozygous for $r_{\lambda_1}lo$ and Ig-1^a were immunized i.p. with 100-μg B1355 in PBS. 7 days later they were bled, and the immune sera were titered by hemagglutination and subsequently analyzed for the magnitude of their antibody response to the α(1,3) and α(1,6) determinants of B1355 as well as for the light chain class used in the response to each determinant by a two-stage RIA.

The $\lambda_1 v_{H\lambda_1}^{\alpha(1,3)+}$ Gene Products of $r_{\lambda_1}+$, Ig-1^a and $r_{\lambda_1}lo$, Ig-1^a Homozygotes have Indistinguishable Ligand-Modifiable Idiotypes, i.e. Combining Sites. The $r_{\lambda_1}+$ and $r_{\lambda_1}lo$ allele might encode two different v_{λ_1} sequences with different amino acids in positions which contribute to the binding site specificity. The $r_{\lambda_1}lo$, Ig-1^a homozygotes which express infrequent high magnitude responses of the α(1,3) specificity produce an antibody which, like all $r_{\lambda_1}+$, Ig-1^a homozygotes, possesses the λ_1 light-chain as its predominant component (Table III). The idiotypes of these antibodies were compared by RIA for their ability to quantitatively inhibit the binding of ^{125}I-labeled J558, a $\lambda_1 v_{H\lambda_1}^{\alpha(1,3)}$ plasmacytoma antibody with α(1,3) specificity, to a purified, ligand-modifiable, anti-idiotypic antibody. The results (Fig. 4) indicate that the combining site idiotypes of the $\lambda_1^{lo} v_{H\lambda_1}^{\alpha(1,3)}$ and $\lambda_1^{+} v_{H\lambda_1}^{\alpha(1,3)}$ antibodies are indistinguishable. That small changes in either the v_{λ_1} or $v_{H\lambda_1}^{\alpha(1,3)}$ contribution to the combining site specificity are detectable by this assay is suggested by several findings. First, it has been shown that reconstruction of a nongerm-line λ_1 light chain possessing amino acid substitutions in complementarity-determining regions with a $v_{H\lambda_1}^{\alpha(1,3)}$ heavy chain can produce an α(1,3) antibody whose altered combining site can be discriminated by this kind of assay (7). Second, this assay discriminates between J558 and MOPC 104E idiotypes (Fig. 4). These plasmacytoma antibodies with α(1,3) specificity have identical λ_1 light chains with the germ-like sequence and apparently closely related $v_{H\lambda_1}^{\alpha(1,3)}$ amino acid sequences which are identical through the 30 amino-terminal residues which have been sequenced (L. Hood, personal communication).

The r_{λ_1} Locus Exerts its Effect in the Stem Cell before B-Cell Expression. The stage of B-cell differentiation at which the r_{λ_1} locus exerts its effect was determined by enumerating λ_1-bearing cells in spleens of $r_{\lambda_1}lo$ and $r_{\lambda_1}+$ homozygotes by staining cell surface

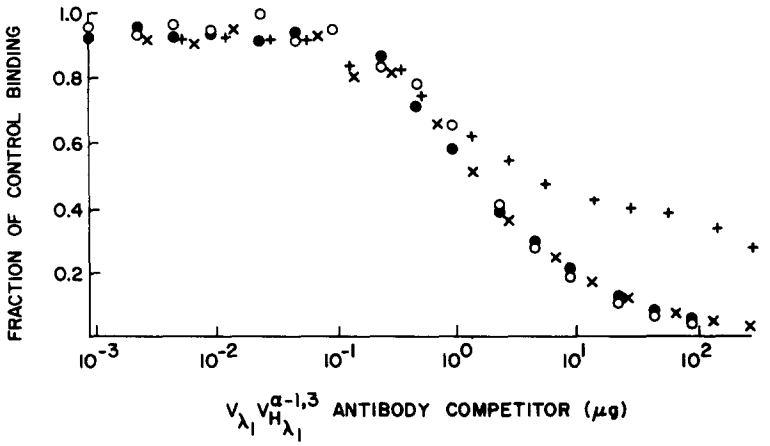

FIG. 4. The $\lambda_1 v_{H\lambda_1}^{\alpha(1,3)+}$ encoded $\alpha(1,3)$ antibodies of $r_{\lambda_1}+$, Ig-1^a and r_{λ_1}lo, Ig-1^a homozygotes possess indistinguishable ligand-modifiable idiotypes. Immune sera from individual (BALB/c × SJL)F_3 mice homozygous for $r_{\lambda_1}+$, Ig-1^a or r_{λ_1}lo, Ig-1^a which gave a high magnitude response to the $\alpha(1,3)$ glucosyl determinant in the λ_1 light chain class were adjusted to the same $\alpha(1,3)$ antibody concentration. They were then serially diluted, and used as competitors in the RIA for the $v_{\lambda_1} v_{H\lambda_1}^{\alpha(1,3)}$ combining site idiotype. J558 (λ_1IgA anti-$\alpha(1,3)$) and MOPC 104E (λ_1IgM anti-$\alpha(1,3)$) were adjusted to the same concentration, serially diluted, and used as competitors in a RIA for the $v_{\lambda_1} v_{H\lambda_1}^{\alpha(1,3)}$ combining site idiotype. The anti-idiotypic antibody in the solid phase recognizes cross-reactive determinants of the J558 and MOPC 104E idiotypes. The ^{125}I-labeled probe is J558. Competition by ○ r_{λ_1}lo $v_{H\lambda_1}^{\alpha(1,3)}$ antibody, ● $r_{\lambda_1}+$ $v_{H\lambda_1}^{\alpha(1,3)}$ antibody, × unlabeled J558, + unlabeled MOPC 104E.

TABLE IV

Frequency of Splenic Lymphocytes Expressing κ- or λ_1-Light Chains

	Percent viable cells staining with:	
	Anti-κ	Anti-λ_1
BALB/c	43 (65/152)*	0.60 (6/960)
(BALB/c × SJL)F_1	—‡	0.24 (5/2,080)
SJL	59 (136/231)	0.018 (2/1.14 × 10^4)

Spleen cells pooled from five individual BALB/c, (BALB/c × SJL)F_1, or SJL mice were stained with FITC-conjugated anti-κ or anti-λ_1. Dead cells were identified by ethidium bromide staining. Viable lymphocytes stained with either FITC-conjugated anti-light chain were enumerated by examining each field at 945-fold magnification using alternate filter combinations.

* (Viable, FITC-positive cells/total viable cells examined).

‡ Not done.

immunoglobulins with a fluorescent anti-λ_1 reagent. The frequency of λ_1-bearing cells in the spleens of r_{λ_1}lo/r_{λ_1}lo homozygotes is reduced nearly 30-fold compared to $r_{\lambda_1}+/r_{\lambda_1}+$ homozygotes, while the number of κ-bearing spleen cells is comparable in both (Table IV). $r_{\lambda_1}+/r_{\lambda_1}$lo heterozygotes have about one-half as many λ_1-bearing cells as $r_{\lambda_1}+/r_{\lambda_1}+$ homozygotes. The results can be used to argue that the r_{λ_1} locus exerts its effect during a stage of B-cell differentiation which precedes the appearance of antigen-sensitive B cells (Discussion).

Discussion

The r_{λ_1} Locus is Linked to (or Identical with) the v_{λ_1} or c_{λ_1} Structural Gene. The primary measurable effect of the r_{λ_1}lo allele is to reduce the frequency of λ_1 ASCs by a factor of 50. However, these λ_1 ASCs expressed by r_{λ_1}lo homozygotes are functionally

equivalent to those expressed by $r_{\lambda_1}+$. Thus, for instance, about 30% of r_{λ_1} lo, Ig-1^a homozygous individuals give a primary λ_1 anti-α(1,3) dextran response of a magnitude and idiotype indistinguishable from $r_{\lambda_1}+$, Ig-1^a homozygotes (Figs. 3 and 4). In the heterozygous r_{λ_1}lo/$r_{\lambda_1}+$ there are one-half as many λ_1 ASCs as in homozygous $r_{\lambda_1}+$ individuals, i.e. there is a gene dosage effect.

This effect of the r_{λ_1}lo allele cannot be accounted for at the level of protein structure, translocation, or transcription. Any such explanation would predict a functional impairment of every cell expressing λ_1. For example, the r_{λ_1}lo allele cannot act at the level of efficiency of induction or of function of the induced plasmacytes. Such effects would be revealed by finding a normal number of λ_1 ASCs induced to become plasmacytes inefficiently. Since this is not found the r_{λ_1}lo allele must control an event which manifests itself at the DNA level. In an r_{λ_1}lo homozygote, the normal expression of λ_1 in a rare ASC could be due either to somatic reversion of a germ-like mutation or to regulation.

First, what kinds of somatic mutational events could account for the r_{λ_1}lo phenotype? The r_{λ_1}lo allele could be either a single-base mutation in a regulatory element controlling translocation or transcription, a nonsense mutation in the r_{λ_1} structural gene which results in a premature termination of λ_1 light chain translation, or a missense mutation in the v_{λ_1} structural gene which could conceivably destroy the ability of the v_{λ_1} product to form a functional domain with any v_H product. In all these examples, the observed expression of the λ_1 light chain in r_{λ_1}lo homozygotes is accounted for by somatic mutational reversion to wild-type in the stem-cell population. The reversions would not necessarily be detectable as changes in the amino acid sequences of λ_1. As examples, a reversion in a mutant regulatory element would not be translated during λ_1 light chain synthesis. A reversion in a nonsense codon in that region of DNA encoding the λ_1 amino-terminal precursor piece would be translated but not detected because it is cleaved from the λ_1 light chain (8).

We can approximate (9) what the mutation rate must be to generate the steady-state number of λ_1-bearing ASCs enumerated by fluorescent anti-λ_1 cell surface staining of r_{λ_1}lo homozygote splenic lymphocytes. If N is the number of rapid-dividing, short-lived cells which generate the immune system, μ is the number of divisions these cells undergo per day, and a is the probability of a mutation at the appropriate base pair during each cell division, then functional revertants will be produced at a daily rate given by $dM/dt = 0.69\, a\mu N$. In the mature immune system of a r_{λ_1}lo homozygote there are $\sim 10^4$ λ_1 ASCs (Table V), and the immune system appears in $t \sim 20$ days, so $dM/dt \sim 5 \times 10^2$ revertants/day. N is taken to be on the order of 10^7 cells, since the steady-state adult B lymphocyte population is no larger than 2×10^8 cells. Thus, a the mutation rate/base pair/division would have to be approximately 2.5×10^{-5}. Since, the absence of repair, the maximum rate per base pair per division for bacterial systems has been reported to be 10^{-7} (discussed in [10]), this class of models seems unlikely.

Second, what kinds of regulatory mechanisms could account for the r_{λ_1}lo allele? The fact that in r_{λ_1}lo/$r_{\lambda_1}+$ heterozygotes the number of r_{λ_1} ASCs is one-half of that found in $r_{\lambda_1}+/r_{\lambda_1}+$ requires that the r_{λ_1} regulatory gene be expressed allelically excluded like the v_{λ_1} structural gene. If it were unlinked to the λ_1 structural gene it is unlikely that it would be expressed allelically excluded. Hence, we assume that it is expressed in *cis* to the λ_1 structural gene and is linked to it. As a consequence of allelic exclusion, the r_{λ_1}lo/$r_{\lambda_1}+$ heterozygote would express only one of the parental loci

TABLE V

A Summary of the Effect of the $r_{\lambda_1}+$ and $r_{\lambda_1}lo$ Alleles on the λ_1 ASC Population and on the Expression of λ_1 Immunoglobulin

Inbred strain	Genotype		Total λ_1 ASCs/ spleen*	v_{λ_1} $v_H^{\lambda_1^3}$ ASCs/ spleen‡	λ_1ASCs§	Relative ratio λ_1ASCs (SJL = 1)	λ_1Ig (% total Ig)	Relative ratio λ_1:κ-light chain (SJL = 1)
					%			
BALB/c	$r_{\lambda_1}+r_{\lambda_1}+$	$v_H^{\lambda_1^3+}/v_H^{\lambda_1^3+}$	2.5×10^6	2.5×10^3	1.4	52	1.5	54
(BALB/c × SJL)F_1	$r_{\lambda_1}+/r_{\lambda_1}lo$	$v_H^{\lambda_1^3+}/v_H^{\lambda_1^3-}$	10^6	—	—	21§	—	23‖
SJL	$r_{\lambda_1}lo/r_{\lambda_1}lo$	$v_H^{\lambda_1^3-}/v_H^{\lambda_1^3-}$	10^4	—	0.027	1	0.028	1
SJA_9	$r_{\lambda_1}lo/r_{\lambda_1}lo$	$v_H^{\lambda_1^3+}/v_H^{\lambda_1^3+}$	—	10^2	—	—	—	—

* Calculated from Table IV, assuming 5×10^7 ASCs/spleen.

‡ Based on the estimate of 1 $v_H^{\lambda_1^3}$ gene/10^2 total v_H gene (18).

§ Calculated from Table IV, assuming that all ASCs stain with one of the FITC-conjugated κ- or λ_1 antibodies. (BALB/c × SJL)F_1 are assumed to have the same number of κ-ASCs as BALB/c.

‖ Data from Table I. (BALB/c × SJL)F_1 are assumed to have the same amount of κ-Ig in normal serum as BALB/c.

— not done.

randomly in each ASC. The result would be a gene dosage effect because the stem cell expressing the $r_{\lambda_1}lo$ allele would have a low probability of becoming a functional λ_1 ASC while the stem cell expressing the $r_{\lambda_1}+$ allele would have a high probability of becoming a functional λ_1 ASC.

What Regulatory Function Might the r_{λ_1} Locus Encode? An acceptable model must account for the finding that, in $r_{\lambda_1}lo$ homozygotes, the $r_{\lambda_1}lo$ allele greatly reduces the number of λ_1 ASCs but the few λ_1 ASCs present express a functional λ_1 light chain indistinguishable from wild-type. In one such molecular level model the $r_{\lambda_1}lo$ allele represents a single base change in a DNA palindrome involved in the translocation event which makes the 5′-end of the v_{λ_1} gene and the 3′-end of the intron-c_{λ_1} gene contiguous (11–13). This single base change would reduce the probability that a DNA endonuclease will nick one of the palindrome sites required for successful translocation.

Consider a heterozygous stem cell, $r_{\lambda_1}+/r_{\lambda_1}lo$, which is undergoing differentiation to a B cell expressing the λ_1 light chain. Initially the stem cell undergoes alleleic exclusion and translocation of v_{λ_1} to intron-c_{λ_1}. If the $r_{\lambda_1}+$ allele is expressed the palindromic sequences are nicked with 100% probability and successful translocation occurs. If the $r_{\lambda_1}lo$ allele is expressed, endonuclease nicking and translocation is successful with only 2% probability. The unsuccessful cells committed to λ_1 expression never synthesize a light chain and thus never express an immunoglobulin receptor. Consequently, the overall probability that translocation in the heterozygote will occur successfully is 51%. The ratio of antigen-sensitive cells which express λ_1 in $r_{\lambda_1}+/r_{\lambda_1}+$, $r_{\lambda_1}+/r_{\lambda_1}lo$, and $r_{\lambda_1}lo/r_{\lambda_1}lo$ mice should be 100:51:2, respectively, and that is the case (Table V). Once a cell has undergone successful translocation and expresses λ_1, whether it is $r_{\lambda_1}lo$ or $r_{\lambda_1}+$, it functions equally well.

In this regard, it should be noted that although the λ_1 and λ_2 light chain class are probably each coded for by one v and one c gene, the λ_1:λ_2 ratio in normal Ig is 4:1 (14). The difference in expression could be accounted for by a difference in the efficiency of translocation for λ_1 and λ_2. Such a model would predict that the 5′-v or the 3′-intron-c palindromes are different for λ_1 and λ_2, but they use the same endonuclease. We are aware of alternative explanations. For example, it may be that λ_1 and λ_2 are translocated at the same frequency and expressed with a different average family of v_Hs in ASCs, and that the expression of λ_1 with its v_H repertoire

TABLE VI

The Distribution in the Magnitude of the Anti-α(1,3) Response in the λ_1 Class of r_{λ_1}lo $v_{H\lambda_1}^{\alpha 1,3^+}$ Homozygotes Fits a Poisson Distribution

Fraction of sample with an actual number of responding units, *r*	Predicted distribution for an average number of responding units, m = 1.6	Observed distribution for the magnitude of $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$ responsiveness*	
r =	Fraction of sample	Fraction of sample	Response range
			μg/ml
0	0.20	0.21 (5/24)	0 (<5)
1	0.32	0.33 (8/24)	5–150
2	0.26	0.25 (6/24)	151–300
3	0.14	0.13 (3/24)	301–450
4	0.05	0.04 (1/24)	450–600
5	0.02	<0.04 (0/24)	>600

*Data from Fig. 3B.

may produce more antigen-binding specificities than the corresponding $\lambda_2 v_H$ combinations. In that case the 4:1 ratio of λ_1/λ_2 in normal Ig would reflect a preferential antigenic selection for $\lambda_1 v_H$ combinations.

The Regulation of a Specific Antibody Response in the λ_1 Class by the r_{λ_1} Locus. In animals possessing the responder heavy chain locus ($v_{H\lambda_1}^{\alpha(1,3)^+}$), the r_{λ_1}lo homozygotes shows wide fluctuations in the magnitude of the anti-α(1,3) dextran response, while the r_{λ_1}+ homozygote shows a uniform, high λ_1 anti-α(1,3) dextran response. Since the effect of the r_{λ_1}lo allele is to reduce the number of λ_1-bearing ASCs, it is reasonable to assume that the variability in the response of r_{λ_1}lo $v_{H\lambda_1}^{\alpha(1,3)}$ homozygotes is due to a large variance in the distribution of ASCs expressing $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$ per individual. Since it was found that the r_{λ_1}lo allele reduces the number of $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$ ASCs such that there exist responder and nonresponder individuals, one can assume that the fluctuation follows a Poisson distribution and calculate the average number of responding units per individual.

If the average number of responding units per individual is *m*, then the probability, *P*, that any individual will actually possess a particular number of responding units, *r*, is given by the Poisson distribution $p(r) = \frac{me^{-m}}{r!}$. The value of *m* for r_{λ_1}lo $v_{H\lambda_1}^{\alpha(1,3)}$ homozygotes can be calculated from the fraction of the sample that fail to give a $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$ primary response to α(1,3) dextran (Fig. 3). The fraction of nonresponders, r_O, is 0.208. For r_O, $p(r_O) = e^{-m}$, so the average number of responding units per individual m = 1.6. If it is assumed that within limits, the level of antibody in a 7-day primary response is proportional to the number of responding units that animal possesses at the moment of immunization, then the response in individuals which respond may be compared to the theoretical distribution expected when m = 1.6. For this comparison the magnitude of the responses was divided into five equal ranges, and the fraction of the total sample which fell within each range was computed. The actual distribution in the magnitude of responsiveness fits the predicted Poisson distribution for m = 1.6 quite closely (Table VI).

The number of ASCs which express $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$ and correspond to one responding unit may be estimated by dividing the number of $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$ ASCs in r_{λ_1}lo homozygotes

(Table V) by the average number of responding units. Hence, one responding unit corresponds to $(10^2 + 1.6) \sim 50$ ASCs. Since the total number of λ_1 ASCs is 50-fold higher in wild-type $r_{\lambda_1}+$ homozygotes than in $r_{\lambda_1}lo$ (Table V), both the number of responding units and also the number of ASCs for $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$ should be increased 50-fold. Thus, for the wild-type, $m = (1.6 \times 50) \sim 80$ responding units and there are (80 responding units × 50 ASCs/responding unit) $\sim 4 \times 10^3$ ASCs expressing $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$. In fact, over 100 $r_{\lambda_1}+v_{H\lambda_1}^{\alpha(1,3)^+}$ individuals have been immunized with α(1,3) dextran (Fig. 3, partial data shown) and no nonresponders have been found.

If the number of wild-type $v_{\lambda_1}v_{H\lambda_1}^{\alpha(1,3)}$ ASCs is typical of any particular germ-line v_Lv_H combination and there are about 10^4 v_Lv_H germ-like combinations (15), then the total number of ASCs per individual would be $(4 \times 10^3)(10^4) \sim 4 \times 10^7$. Of 4×10^7 ASCs with germ-line encoded v_Lv_H, only about 4×10^5 will express specificities selected upon during germ-line evolution. The others are starting points for somatic evolution (16).

The Regulation of Normal Immunoglobulin in the λ_1 Class by the r_{λ_1} Locus. Another phenotypic effect of the r_{λ_1} locus which must be accounted for in terms of different numbers of λ_1-bearing ASCs in the regulation of the level of λ_1 associated with normal immunoglobulin in $r_{\lambda_1}+$ and $r_{\lambda_1}lo$ homozygotes. The analysis rests on two observations. The first observation is that for $r_{\lambda_1}+$ homozygotes the ratio of λ_1:κ-bearing ASCs determined by spleen cell staining with fluorescent labeled anti-κ or anti-λ_1 is the same as the ratio of λ_1:κ light chains associated with normal immunoglobulin (Table V).

Thus, in $r_{\lambda_1}+$ homozygotes the observed fraction of ASCs which express λ_1 is 1/70 and the observed fraction of λ_1 light chain in normal serum is 1/65. The second observation is that the ratio of the v_{λ_1} gene to the total v_L gene pool has been estimated by an independent analysis of sequences to have a mean value of ~1/100 (1/60–1/260) (15).

The ratio of λ_1/κ in virgin ASCs represents the probability of expression of the v_{λ_1} gene relative to the total v_L pool. Since this ratio is the same as the ratio of v_{λ_1}/v_κ germ-line genes, the probability of expression of each v_L gene is, on an average, the same. This means that the $r_{\lambda_1}+$ regulatory gene operates with the same efficiency as an average $r_\kappa+$ regulatory gene. The ratio of λ_1/κ in serum Ig represents the probability of induction of a given λ_1 ASC relative to a κ-ASC. Since this ratio is the same as the ratio of λ_1/κ ASCs, the probability of induction by the immunogenic universe is the same for any given class of ASC defined as expressing a unique V_L framework (subgroup) (15, 16). The $r_{\lambda_1}lo$ mutation (as well as the $r_{\lambda_1}lo$-like behavior of the λ_2 gene) stresses that for any given germ-line v gene a subtle modulation of the frequency of its expression can be imposed by evolutionary selection. This is why we discuss the above ratios by referring to an average efficiency expression of germ-line v_κ-genes.

The gene dosage expression of λ_1 poses a paradox, for it is not immediately obvious that the level of serum λ_1Ig should depend strictly on the number of ASCs initially expressing λ_1. The ratio of λ_1-bearing ASCs in $r_{\lambda_1}lo/r_{\lambda_1}lo$:$r_{\lambda_1}lo/r_{\lambda_1}+$:$r_{\lambda_1}+/r_{\lambda_1}+$ genotypes is the same as the ratio of λ_1 light chains associated with normal immunoglobulin in each genotype, and is approximately 1:25:50. Clearly, the $v_{\lambda_1}v_H$ repertoires in a $r_{\lambda_1}+/r_{\lambda_1}+$ homozygote and a $r_{\lambda_1}lo/r_{\lambda_1}+$ heterozygote must be the same. If feedback inhibition by antibody itself limits the level of a response any gene dosage effect should be masked.

One solution might be considered. The response to an immunogen of any given class of ASC (defined by the v_L framework it expresses) depends on the number of mutational steps it must undergo to recognize the antigen. Those requiring few steps are more likely to respond. As the number of a given class of ASC is reduced the total number of possible somatic derivatives generated from it is reduced. In such a situation, an ASC class which might require more mutational steps becomes competitive in response to an immunogen. Thus, in the r_{λ_1}lo homozygotes, ASCs expressing various $v_\kappa v_H$ combinations replace the lost ASCs which would have expressed $v_{\lambda_1} v_H$ combinations. These $v_\kappa v_H$ ASC replacements become inducible by the determinants in the antigenic universe which preferentially would have induced their missing $v_{\lambda_1} v_H$ counterparts. This conclusion is supported by a study (17) with the κ-locus in rabbits. Suppression of the expression of κ by the use of anti-allotypic sera leads to a compensatory increase in the level of immunoglobulin bearing a light chain lacking the given allotypic marker (probably λ).

Is the Regulatory Gene Marked by the r_{λ_1}lo Allele an Example of an Element Controlling the Expression of an Entire Light Chain (Translocon) or of a Single v-Gene? Since the λ_1 translocon consists of only one v-gene, this question is unresolved. Given that the ratio of λ_1/κ ASC, serum Ig, and germ-line v-genes is ~1/100, two modes of expression of v_L-genes are reasonable. If the r_{λ_1} gene regulates expession of the λ_1 translocon then the stem cell first decides which translocon to activate, λ_1 or κ, after which the activated translocon expresses sequentially all of its v-genes. Such a mode of expression would make the ratio of λ_1/κ ASCs equal the v_{λ_1}/v_κ germ-line gene ratio only if each translocon were initially chosen with equal probability and if subsequently each translocon could count its v-gene repertoire by expressing sequentially each v-gene in the translocon at each cell division. The r_{λ_1}lo allele could act to reduce the probability that the λ_1 translocon was chosen for activation. The *bas* mutation in rabbits (18), which results in the specific loss of the κ-light-chain class, might represent a defect in translocon expression.

Alternatively, if the r_{λ_1} gene regulates expression of a unique v-gene then the stem cell could simply activate v_L-genes randomly, treating v_{λ_1} and v_κ indifferentially. This latter mode of expression also would make the λ_1/κ ratio in ASCs equal to the v_{λ_1}/v_κ germ-line gene ratio. The model for the action of the r_{λ_1}lo allele presented earlier exemplifies regulation of a unique v-gene. While regulation of translocon expression or individual v-gene expression has been presented as alternative interpretations of the r_{λ_1}lo allele, it is probable that both types of regulatory genes operate and that we have revealed by the r_{λ_1}lo allele only one of them.

Do Regulatory Genes of the r_{λ_1}-Type Operate to Control the Expression of the Heavy Chain Class? Only with special assumptions is our analysis applicable to the heavy chain translocon because, unlike the light chain translocon, the same set of v_H-genes is expressed with a family of some 10 c_H-genes. The general rule is that the suppression of one allele does not lead to a compensatory increase in the expression of the other allele. This has been shown for murine λ_1 (this paper), rabbit-κ (17) and murine γG_{2a} (19). If there is a compensatory increase it appears to come from the other classes, κ for murine λ_1, λ for rabbit κ and an unknown γG class for murine γG_{2a}. This implies that the choice of class is determined by regulatory genes functioning in the B cell itself and not by genes regulating intercellular events, e.g. effective level of cooperative activity, secretion of hormones, homing patterns, etc. In other words, the heavy chain

class which a given B cell will be committed to express is determined by regulatory genes acting intracellularly, not by those acting intercellularly; the assumption being that a selective intercellular mechanism determining class would be unable to distinguish c_H-alleles. Normally, the level would be determined by the proportion of B cells committed to express a given allele or class of heavy chain. The intercellular signals for commitment (including the antigen-dependent ones) determine the frequency of the switch from [IgM + IgD] to other classes (20), not which class is expressed. Once switched, induction by antigen selects B cells on the basis of their combining site, not the class of immunoglobulin they express. This argument is strongest for the IgG classes and the extent to which it can be applied to IgA and IgE is open.

The existence of such an intracellularly acting regulatory gene has been implied by several studies (21) in which the level of a given class of heavy chain is associated with the allotype. This suggests both linkage and *cis* action between the regulatory gene and the structural gene determining allotype. If our assumptions are correct, then the expression of both heavy and light chain classes have in common an intracellular mechanism involving a regulatory gene of the r_{λ_1}-type.

Summary

We describe here two alleles, an allele of the λ_1 locus present in the SJL strain (r_{λ_1}lo) and an allele of the λ_1 locus present in the BALB/c strain ($r_{\lambda_1}+$), of a regulatory gene locus which specifically influences the expression of the mouse λ_1 light chain structural gene. The r_{λ_1} regulatory gene is not linked to either the major histocompatibility complex or to the heavy-chain allogroup but appears to be linked to the λ_1 structural gene locus.

In the homozygous state, the present of the r_{λ_1}lo allele results in a 50-fold reduction in the number of λ_1 antigen-sensitive, bone-marrow derived lymphocytes (ASCs) compared to the presence of the $r_{\lambda_1}+$ allele. However, those few λ_1ASCs present in r_{λ_1}lo homozygotes can be induced normally to produce λ_1 light chains indistinguishable from those found in $r_{\lambda_1}+$ homozygotes. The reduction in λ_1ASC's due to the r_{λ_1}lo allele results both in a reduction in the amount of λ_1 Ig in the serum and also in a large variation in the magnitude of the λ_1 antibody response to $\alpha(1,3)$ dextran by individual animals. This variation permits the estimate that, on the average, 50 B cells of anti-$\alpha(1,3)$ specificity must be present per animal to permit a measurable response.

Surprisingly, the expression of a gene locus regulating λ_1 light chain expression (r_{λ_1} locus) shows a clear gene dosage effect with r_{λ_1}lo/$r_{\lambda_1}+$ heterozygotes having ½ the number of λ_1ASCs and ½ the amount of serum λ_1 Ig as $r_{\lambda_1}+/r_{\lambda_1}+$ homozygotes. This fact permits an analysis of the relationship between germ-line v-genes and their individual expression in serum Ig.

The r_{λ_1} locus controls specifically a DNA-level event which occurs in stem cells as they become committed to λ_1 light chain expression. We postulate that the r_{λ_1} locus represents one of the DNA level recognition sites involved in the translocation event which places the v_{λ_1} and c_{λ_1} structural genes in a transcriptional unit.

Received for publication 7 June 1978.

References

1. Brack, C., and S. Tonegawa. 1977. The V- and C-parts of the light chain gene of a mouse myeloma cell are 1250 non-translated bases apart. *Proc. Natl. Acad. Sci. U. S. A.* **74:**5652.
2. Gilmore-Hebert, M., and R. Wall. 1978. Immunoglobulin light chain mRNA is processed from large nuclear RNA. *Proc. Natl. Acad. Sci. U. S. A.* **75:**342.
3. Milstein, C., G. G. Brownlee, E. M. Cartwright, J. M. Jarvis, and N. J. Proudfoot. 1974. Sequence analysis of immunoglobulin light chain messenger RNA. *Nature (Lond.).* **252:**354.
4. Blomberg, B., W. R. Geckeler, and M. Weigert. 1973. Genetics of the antibody response to dextran in mice. *Science (Wash. D. C.).* **177:**178.
5. Geckeler, W. R. 1977. On the regulation of expression of the murine λ_1 light chain. University Microfilms International, Ann Arbor, Mich.
6. Staats, J. 1976. Standardized nomenclature for inbred strains of mice: sixth listing. *Cancer Res.* **36:**4333.
7. Carson, D., and M. Weigert. 1973. Immunochemical analysis of the cross-reacting idiotypes of mouse myeloma proteins with anti-dextran activity and normal anti-dextran antibody. *Proc. Natl. Acad. Sci. U. S. A.* **70:**235.
8. Schecter, I., and Y. Burstein. 1976. Identification of N-terminal methionine in the precursor of immunoglobulin light chain. Initiation of translation of messenger ribonucleic acid in plants and animals. *Biochem. J.* **153:**543.
9. Cohn, M. 1973. Antibody diversification: the somatic mutation model revisited. *In* The Biochemistry of Gene Expression in Higher Organisms. J. K. Pollack and J. W. Lee, editors. Reidel, Dordrecht, Holland, 574.
10. Mäkelä, and A. M. Cross. 1970. The diversity and specialization of immunocytes. *Prog. Allergy.* **14:**145.
11. Tonegawa, S., N. Hozumi, G. Matthyssens, and R. Schuller, 1976. Somatic changes in the content and context of immunoglobulin genes. *Cold Spring Harbor Symp. Quant. Biol.* **41:**877.
12. S. Tonegawa, A. M. Maxam, R. Tizard, O. Bernard, and W. Gilbert. 1978. Sequence of a mouse germ-line gene for a variable region of an immunoglobulin light chain. *Proc. Natl. Acad. Sci. U. S. A.* **75:**1485.
13. Wuilmart, C., J. Urbain, and D. Givol. 1977. On the location of palindromes in immunoglobulin genes. *Proc. Natl. Acad. Sci. U. S. A.* **74:**2526.
14. Blaser, K., and H. N. Eisen. 1978. Lambda$_2$ light chains in normal mouse immunoglobulins. *Proc. Natl. Acad.Sci. U. S. A.* **75:**1495.
15. Weigert, M., and R. Riblet. 1976. Genetic control of antibody variable regions. *Cold Spring Harbor Symp. Quant. Biol.* **41:**837.
16. Cohn, M., B. Blomberg, W. Geckeler, W. Raschke, R. Riblet, and M. Weigert. 1974. First order considerations in analyzing the generator of diversity. *In* The Immune System: Genes, Receptors, and Signals. F. Fox, editor Academic Press, Inc., New York.
17. Dubiski, S. 1967. Suppression of synthesis of allotypically defined immunoglobulins and compensation by another subclass of immunoglobulin. *Nature (Lond.).* **214:**1365.
18. Kelus, A. S., and S. Weiss. 1977. Variant strain of rabbits lacking immunoglobulin κ polypeptide chain. *Nature (Lond.).* **256:**156.
19. Jacobson, E. B., and L. A. Herzenberg, 1972. Active suppression of immunoglobulin allotype synthesis. 1. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL × BALB/c)F_1 mice. *J. Exp. Med.* **135:**1151.
20. Coffman, R., and M. Cohn. 1977. The class of surface immunoglobulin on virgin and memory B lymphocytes. *J. Immunol.* **118:**1806.
21. Waltzer, P. D., and H. G. Kunkel. 1974. The correlation of serum IgD concentration with Gm allotype. *J. Immunol.* **113:**274.