ON A REGULATORY GENE CONTROLLING THE EXPRESSION 
OF THE MURINE λ1 LIGHT CHAIN*

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The λ1 light chain is encoded by a single germ-line νλ and cλ gene. Upon 
commitment, the νλ gene is translocated in cis to a transcription unit which includes 
the cλ gene separated from νλ by a nontranslated stretch of some 1,250 nucleotides 
(intron) (1). This unit is then transcribed as the high molecular weight nuclear RNA 
(HnRNA) which must be processed by excision of the intron to yield the polysome-
bound mRNA in which the coding of νλ and cλ must be contiguous (2, 3).

The νλ germ-line gene complemented with a set of closely-related heavy chain 
variable region (νH) germ-line genes (referred to as νH[1,3]) encodes the anti-
α(1,3)dextran specificity of high responder strains of mice. The role of the νH[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 λ1 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

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† Abbreviations used in this paper: ASC, antigen-sensitive, bone-marrow derived lymphocytes; BSA, bovine 
serum albumin; cλ, 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, the a allele of Ig-1, which identifies a heavy chain gene complex containing νH[1,3] genes (νH[1,3]α); Ig- 
1, the a allele of Ig-1, which identifies a heavy chain gene complex lacking νH[1,3] genes (νH[1,3]α); κ, the 
murine kappa light chain, defined by the O-terminal tripeptide ALA ASP OYS; λ1, the murine 
lambda-one light chain, defined by the C-terminal tripeptide ALA ASP CYS; λ2, the murine 
lambda-two light chain, defined by the C-terminal tripeptide ALA ASP CYS SER; Λ1, locus, a gene 
locus regulating heavy chain expression; ν1+, an allele of the Λ1 locus present in the BALB/c strain; 
ν1, locus, an allele of the Λ1 locus present in the SJL strain; λ2, the murine lambda-two light chain, defined by 
the C-terminal tripeptide SER LEU SER PRO ALA GLU OYS LEU; NRS, normal rabbit serum; 
PBSAE, phosphate-buffered saline with 10^-3 M sodium azide and 10^-3 M (ethylenedinitrilo)-tetraacetic 
acid; RIA, radioimmunoassay; νκ, variable region of the heavy chain or the structural gene encoding it; 
νH[1,3], one of several germ-line heavy chain variable regions, or the gene encoding it, which complements 
with νλ to produce a combining site with specificity for the α(1,3) glucosyl linkage; νκ, variable region of 
the kappa light chain or the structural gene encoding it; νλ, variable region of the light chain or the 
structural gene encoding it; νλ, variable region of the lambda-one light chain or the structural gene 
encoding it; ν2, variable region of the lambda-two light chain or the structural gene encoding it; 
νλ, νH[1,3], the possession of both a νλ structural gene and also a νH[1,3] set, such that the association of the 
products of the νλ and any one of the νH[1,3] genes produces a combining site with specificity for 
the α(1,3) glucosyl linkage.
fraction of a B1355 antibody response with specificity for each linkage and also the $\kappa:\lambda_1$ ratio of the $\alpha(1,3)$ and $\alpha(1,6)$ antibody response.

B1355, containing $\alpha(1,3)$ and $\alpha(1,6)$ glucosyl linkages, or B512, which contains $\alpha(1,6)$ linkages are the gift of Dr. Allene Jeanes, Department of Agriculture, Peoria, Ill. Each is dissolved to a final concentration of 100 $\mu$g/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 ($\alpha(1,3) + \alpha(1,6)$) and to two tubes with adsorbed B512 ($\alpha(1,6)$ only) such that neither the $\alpha(1,3)$ nor the $\alpha(1,6)$ binding sites are saturated by the anti-dextran antibody (generally <10 $\mu$g). 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 $\kappa$- and $\lambda_1$-bearing immunoglobulins in these complexes is determined. $^{125}$I-labeled anti-$\kappa$, 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-$\lambda_1$ 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-$\kappa$ or anti-$\lambda_1$ 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 $\lambda_1$ anti-dextran/$^{125}$I-labeled anti-$\lambda_1$ is constructed by binding known amounts of purified J558, a $\lambda_1$ plasmacytoma antibody with $\alpha(1,3)$specificity, to B1355 dextran in the first stage, and subsequently measuring the amount of $^{125}$I-labeled anti-$\lambda_1$ antibody bound in the second stage. A standard curve is likewise constructed for bound $\kappa$-anti-dextran/$^{125}$I-labeled anti-$\kappa$ antibody by binding known amounts of purified W3129, a $\kappa$-plasmacytoma antibody with $\alpha(1,6)$specificity, to B512 dextran in the first stage, and subsequently measuring the amount of $^{125}$I-labeled anti-$\kappa$ antibody bound in the second stage. The amount of antibody with $\alpha(1,3)$specificity is calculated by subtracting the amount of antibody bound to $\alpha(1,6)$determinants (B512) from the amount bound to $\alpha(1,3)$ and $\alpha(1,6)$determinants (B1355).

Preparation of Anti-Idiotype Antibody. An anti-idiotype antibody specific for the combining site of anti-$\alpha(1,3)$dextran antibodies encoded by $\nu_{\lambda_1}^{\nu_{\lambda_1}}$ was prepared by immunizing rabbits with MOPC 104E ($\lambda_1$ IgM anti-$\alpha(1,3)$dextran). Most of the anti-$\lambda_1$ antibody was removed by affinity chromatography of the anti-serum on Y 5431 ($\lambda_1$ Bence-Jones) coupled to Sepharose 4B. The effluent was chromatographed on a Sepharose 4B column to which J558 ($\lambda_1$ IgA anti-$\alpha(1,3)$dextran), which idiotypically cross-reacts with MOPC 104E, was coupled. The acid eluate of this column was neutralized and passed over a Y5606 ($\lambda_1$ IgG3) Sepharose 4B column to remove residual anti-$\lambda_1$ activity, and the anti-idiotype antibody was obtained in the effluent. The binding of the purified anti-idiotype antibody can be specifically inhibited by $\alpha(1,3)$containing dextrans.

Results

The Amount of $\lambda_1$-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 $\lambda_1$ levels (Table I). Two inbred strains, however, SJL and BSVS, have very low levels of $\lambda_1$. The reduction in $\lambda_1$ serum levels is specific for the $\lambda_1$ light chain class since inbred strains with low or high levels of $\lambda_1$ have comparable levels of the $\kappa$-light chain in normal serum.

To demonstrate that strains with very low measured levels of $\lambda_1$ actually possess a finite amount of $\lambda_1$lg, portions of SJL normal serum were preincubated with anti-$\lambda_1$
### TABLE I

| Mouse strain          | \(\lambda_1\text{lg} \mu\text{g/ml serum} \) | \(\kappa\text{lg} \mu\text{g/ml serum} \) | \(\lambda_1:\kappa\) % |
|-----------------------|--------------------------------|--------------------------------|----------------|
| BALB/c                | 68.0                          | 4,400                           | 1.5*           |
| BALB/c (nu/nu)        | 66.0                          | --                              | --             |
| SJL                   | 2.5                           | 7,000                           | 0.035          |
| SJA\(_b\)             | 0.45                          | --                              | --             |
| BSVS                  | 1.0                           | 3,500                           | 0.028          |
| BRVR                  | 43.0                          | --                              | --             |
| (SJL×BSVS)\(_F1\)     | 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 \(\lambda_1:\kappa\)-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 \(\lambda_1\) (data not shown). We conclude that the low values of \(\lambda_1\text{lg}\) in SJL and BSVS are real and that the \(\lambda_1\) level in BALB/c normal serum is 30-fold higher than in SJL. When a comparison is made of \(\lambda_1\text{lg}\) levels relative to \(\kappa\text{lg}\) levels (the \(\lambda_1:\kappa\)-ratio) the \(\lambda_1\text{lg}\) level is 50-fold higher in BALB/c than in SJL.

The Expression of \(\lambda_1\) is Controlled by a Single Genetic Locus. To analyze the genetics of the control of \(\lambda_1\) expression, (BALB/c × SJL)\(_F1\), \(_F2\), and \(_F3\) progeny were examined. BALB/c has high levels of \(\lambda_1\) and SJL has low levels of \(\lambda_1\). 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-2\(_d\) haplotype and the heavy chain complex identified by the Ig-1\(_a\) allotype. SJL has the H-2\(_a\) haplotype and the Ig-1\(_b\) allotype (6).

(BALB/c × SJL)\(_F1\) progeny are phenotypically \(\lambda_1\) intermediates, i.e., their average \(\lambda_1\) level is one-half that of the \(\lambda_1\) high phenotype (Results).

(BALB/c × SJL)\(_F2\) progeny were typed for their H-2 haplotypes and heavy chain allotypes. The \(_F2\) progeny homozygous for all combinations of H-2 haplotype and heavy chain allotype were tested for \(\lambda_1\) serum levels. The results are shown in Fig.
WILLIAM GECKELER, JEROME FAVERSHAM, AND MELVIN COHN

Fig. 1. The amount of $\lambda_1$ Ig in the serum of (BALB/c × SJL)F2 progeny homozygous for H-2 haplotype and heavy chain allogroup. (BALB/c × SJL)F2 progeny homozygous for H-2 haplotype and heavy chain allogroup were tested for serum levels of $\lambda_1$ Ig by RIA. Individuals with less than 5 µg/ml $\lambda_1$ Ig are phenotypically $\lambda_1$ low and are postulated to possess the $\lambda_1$lo/lo genotype. Individuals with greater than 10 µg/ml $\lambda_1$ Ig are phenotypically $\lambda_1$ intermediate or $\lambda_1$ high, and are postulated to possess the $\lambda_1$hi/hi or $\lambda_1$hi/lo genotype, respectively.

TABLE II
Segregation in the (BALB/c × SJL)F2 Generation of the $\lambda_1$ Locus as a Single Genetic Unit, Unlinked to the Major Histocompatibility Complex (H-2) or to the Heavy Chain Allogroup

| Observed $\lambda_1$ phenotype | Postulated genotype | H-2 hi/hi Ig-a/a | H-2 hi/hi Ig-1/b/b | H-2 hi/hi Ig-1/a/a | H-2 hi/hi Ig-1/b/b |
|-------------------------------|---------------------|------------------|-------------------|------------------|------------------|
| Low                           | $\lambda_1$lo/lo   | 7                | 8                 | 6                | 6                |
| Intermediate to high          | $\lambda_1$lo/hi   | 24               | 26                | 17               | 19               |
|                               | $\lambda_1$hi/hi   | 31               | 34                | 23               | 25               |

An SJL × BALB/c mating comprised the parental generation for the genetic segregation study. SJL mice are homozygous $\lambda_1$lo, H-2a, Ig-a. BALB/c mice are homozygous $\lambda_1$hi, H-2b, Ig-b. 512 F2 progeny were tested for H-2 haplotype and heavy chain allogroup. Individuals homozygous for each combination of H-2 and allotype were then tested for the level of $\lambda_1$ Ig in normal serum. Of 512 mice ¼ 6 (32) are expected to be homozygous at both alleles for any combination of H-2 and heavy chain allogroup. Within each genotype category ¼ are expected to be $\lambda_1$lo/hi, ¼ are expected to be $\lambda_1$lo/hi, and ¼ are expected to be $\lambda_1$hi/hi.

1 and summarized in Table II. Within each group of H-2 and allogroup homozygotes both $\lambda_1$ intermediate to high phenotypes and also $\lambda_1$ low phenotypes were recovered. The ratio of $\lambda_1$ intermediate to high:$\lambda_1$ low phenotypes within each group was 3:1.

The simplest interpretation of the segregation ratios of high and low $\lambda_1$ phenotypes is that two alleles, $\lambda_1$hi and $\lambda_1$lo, at a single genetic locus, $\lambda_1$, control the level of $\lambda_1$ light chain. Thus, BALB/c ($\lambda_1$hi/+) has the $\lambda_1$ high phenotype and SJL ($\lambda_1$lo/lo) has the $\lambda_1$ low phenotype. In the F2, the genotypes $\lambda_1$hi/lo, $\lambda_1$lo/+, $\lambda_1$hi/hi, and $\lambda_1$hi/lo are expected to distribute in a 1:2:1 ratio. It is difficult to distinguish consistently whether an individual is $\lambda_1$ high or $\lambda_1$ intermediate, since the phenotype within each genotype group is obscured by individual variation. On the other hand, it is always possible to distinguish $\lambda_1$ low from $\lambda_1$ high or $\lambda_1$ intermediate...
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 F₂, the rₙ, locus must be unlinked to either the H-2 or the heavy chain gene complexes. Further, the rₙ, locus is not sex-linked.

F₂ 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 rₙ, + or rₙ, lo, were mated. In all cases, mating pairs possessing the highest λ₁ levels (presumed rₙ, +/rₙ, +) yielded F₃ progeny which were all λ₁ high, while mating pairs chosen as rₙ, lo/rₙ, lo yielded F₃ progeny all of which were phenotypically λ₁ low. These results confirm that λ₁ expression breeds true and is controlled by a single genetic locus.

The rₙ, Locus Affects the Antibody Response to the α(1,3) Glucosyl Linkage which is Encoded by the vₐᵥ₉(1,3) Genes. To analyze the mechanism by which the rₙ, locus regulates λ₁ light chain expression, mice homozygous for the Ig-I b allotype and either the rₙ, + or rₙ, lo allele were immunized with B1355. It is known that rₙ, +/rₙ, + mice possessing the v₉(1,3) alleles respond predominantly to the α(1,3) glucosyl linkages of B3155 by producing an antibody encoded exclusively by the complementing genes vₙᵥ₉(1,3) (7). The v₉(1,3) genes are present in the heavy chain gene complex identified by the Ig-I b allotypic marker. The response is uniformly of high magnitude in individuals with the rₙ, +, Ig-I b genotype. BALB/c, one of the parents used in the genetic analysis of the rₙ, locus, possesses this genotype. SJL, the other parent used in the genetic analysis of the rₙ, locus, is v₉(1,3) (linked to Ig-I b) and would be a low responder to B1355, even if it were rₙ, +/rₙ, +.
Fig. 3. Magnitude of the \( \lambda_1 \) anti-\( \alpha(1,3) \) primary response in \( r_\alpha^+, \ Ig-1^a \) and \( r_\alpha^{lo}, \ Ig-1^a \) homozygotes (BALB/c \( \times \) SJL)F3 mice measured by a two-stage RIA. (BALB/c \( \times \) SJL)F3 mice were allotyped and tested for the amount of \( \lambda_1 \lg \) in their serum. Those classified as homozygous for \( r_\alpha^+, \ Ig-1^a \) or \( r_\alpha^{lo}, \ Ig-1^a \) were then immunized i.p. with 100 \( \mu \)g B1355 in PBS and bled 7 days later. Panel A represents the analysis of \( r_\alpha^+, \ Ig-1^a \) homozygotes and panel B represents the analysis of \( r_\alpha^{lo}, \ Ig-1^a \) homozygotes.

(BALB/c \( \times \) SJL)F3 mice, homozygous for the Ig-1\( ^a \) heavy chain allotype and either the \( r_\alpha^+ \) or the \( r_\alpha^{lo} \) allele, were immunized with B1355. Their \( \lambda_1 \)-antibody response to the \( \alpha(1,3) \) glycosyl linkage is shown in Fig. 3. \( r_\alpha^+, \ Ig-1^a \) 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, \( r_\alpha^{lo}, \ Ig-1^a \) homozygotes exhibit great individual variation in the magnitude of their \( \lambda_1 \) anti-\( \alpha(1,3) \) response. From Table III it can be seen that the anti-\( \alpha(1,3) \) response of these \( r_\alpha^{lo} \) homozygotes can be classified into three groups, high, intermediate, and low. The antibody of high responders is predominantly in the \( \lambda_1 \) light chain class. Intermediate responders fall into three classes; those whose response is in the \( \lambda_1 \) light chain class alone, those whose response is in both \( \lambda_1 \) and \( \kappa \)-light chain class, and those whose response is only in the \( \kappa \)-light chain class. The antibody of low responders is predominantly in the \( \kappa \)-class. SJL\( ^a \) mice, which are congenic with SJL except for the Ig-1\( ^a \) allotype, and thus possess \( r_\alpha^{lo} \) 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 \( \alpha(1,3) \) dextran response seen in (BALB/c \( \times \) SJL)F3 mice homozygous for \( r_\alpha^{lo} \) and Ig-1\( ^a \) is unlikely due to genetic loci other than \( \lambda_1 \) which are still segregating. Rather it is due to the \( r_\alpha^{lo} \) allele itself.

The \( r_\alpha^{lo} \) allele generates the wide fluctuation in the magnitude of the \( \alpha(1,3) \) dextran response among \( r_\alpha^{lo} \), Ig-1\( ^a \) homozygotes by a process which acts randomly in each animal. Thus, if one intermates F3 \( r_\alpha^{lo} \), 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).


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**Table III**

*Light Chain Class and Determinant Specificity of the Primary Antibody Response to B1355 in (BALB/c × SJL)F<sub>2</sub> Mice Homozygous for r<sub>α</sub>,lo and Ig-1<sup>a</sup>*

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

(BALB/c × SJL)F<sub>2</sub> mice homozygous for r<sub>α</sub>,lo and Ig-1<sup>a</sup> 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.

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The gene products of r<sub>α</sub>, +, Ig-1<sup>a</sup> and r<sub>α</sub>, lo, Ig-1<sup>a</sup> homozygotes have indistinguishable ligand-modifiable idiotypes, i.e. combining sites. The r<sub>α</sub>, + and r<sub>α</sub>,lo allele might encode two different v<sub>α</sub> sequences with different amino acids in positions which contribute to the binding site specificity. The r<sub>α</sub>,lo, Ig-1<sup>a</sup> homozygotes which express infrequent high magnitude responses of the α(1,3) specificity produce an antibody which, like all r<sub>α</sub>, +, Ig-1<sup>a</sup> homozygotes, possesses the λ<sub>1</sub> 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<sup>I</sup>-labeled J558, a λ<sub>TVH</sub><sup>123</sup> 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 λ<sub>1</sub>TVH<sup>123</sup> and κ<sub>1</sub>VH<sup>123</sup> antibodies are indistinguishable. That small changes in either the v<sub>α</sub> or v<sub>β</sub> 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 non-gem-line λ<sub>1</sub> light chain possessing amino acid substitutions in complementarity-determining regions with a v<sub>β</sub><sup>123</sup> 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<sub>1</sub> light chains with the germ-like sequence and apparently closely related v<sub>β</sub><sup>123</sup> amino acid sequences which are identical through the 30 amino-terminal residues which have been sequenced (L. Hood, personal communication).

The r<sub>α</sub> locus exerts its effect in the stem cell before B-cell expression. The stage of B-cell differentiation at which the r<sub>α</sub> locus exerts its effect was determined by enumerating λ<sub>1</sub>-bearing cells in spleens of r<sub>α</sub>,lo and r<sub>α</sub>, + homozygotes by staining cell surface
Fig. 4. The \( \lambda_1 \) encoded \( \alpha(1,3) \) antibodies of \( r_\lambda +, Ig-1^* \) and \( r_\lambda 0, Ig-1^* \) homozygotes possess indistinguishable ligand-modifiable idiotypes. Immune sera from individual (BALB/c × SJL)F\(_3\) mice homozygous for \( r_\lambda +, Ig-1^* \) or \( r_\lambda 0, Ig-1^* \) which gave a high magnitude response to the \( \alpha(1,3) \) glucosyl determinant in the \( \lambda_1 \) 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 \( V_{\lambda_1} V_{\alpha-1,3} \) combining site idiotype. J558 (\( \lambda_1 \)IgA anti-\( \alpha(1,3) \)) and MOPC 104E (\( \lambda_1 \)IgM anti-\( \alpha(1,3) \)) were adjusted to the same concentration, serially diluted, and used as competitors in a RIA for the \( V_{\lambda_1} V_{\alpha-1,3} \) 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 \( O r_\lambda 0 V_{\alpha-1,3} \) antibody, \( \bullet r_\lambda + V_{\alpha-1,3} \) antibody, \( x \) unlabeled J558, \( + \) unlabeled MOPC 104E.

TABLE IV

| Percent viable cells staining with: | Anti-\( \kappa \) | Anti-\( \lambda_1 \) |
|-----------------------------------|----------------|----------------|
| BALB/c                            | 43 (65/152)*  | 0.60 (6/960)  |
| (BALB/c × SJL)F\(_1\)             |                |                |
| 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-\( \kappa \) or anti-\( \lambda_1 \). 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.

Discussion

The \( r_\lambda \) Locus is Linked to (or Identical with) the \( r_\kappa \) or \( \alpha_\lambda \) Structural Gene. The primary measurable effect of the \( r_\lambda 0 \) allele is to reduce the frequency of \( \lambda_1 \) ASCs by a factor of 50. However, these \( \lambda_1 \) ASCs expressed by \( r_\lambda 0 \) homozygotes are functionally
equivalent to those expressed by \( r_\lambda^+ \). Thus, for instance, about 30% of \( r_\lambda^+ \), Ig-1a homozygous individuals give a primary \( \lambda_1 \) anti-\( \alpha(1,3) \) dextran response of a magnitude and idiotype indistinguishable from \( r_\lambda^+ \), Ig-1a homozygotes (Figs. 3 and 4). In the heterozygous \( r_\lambda^+ / r_\lambda^+ \) there are one-half as many \( \lambda_1 \) ASCs as in homozygous \( r_\lambda^+ \) individuals, i.e. there is a gene dosage effect.

This effect of the \( r_\lambda^+ \) 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 \( \lambda_1 \). For example, the \( r_\lambda^+ \) 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 \( \lambda_1 \) ASCs induced to become plasmacytes inefficiently. Since this is not found the \( r_\lambda^+ \) allele must control an event which manifests itself at the DNA level. In an \( r_\lambda^+ \) homozygote, the normal expression of \( \lambda_1 \) 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 \( r_\lambda^+ \) phenotype? The \( r_\lambda^+ \) allele could be either a single-base mutation in a regulatory element controlling translocation or transcription, a nonsense mutation in the \( \lambda_1 \) structural gene which results in a premature termination of \( \lambda_1 \) light chain translation, or a missense mutation in the \( \nu_\lambda \) structural gene which could conceivably destroy the ability of the \( \nu_\lambda \) product to form a functional domain with any \( \nu_\kappa \) product. In all these examples, the observed expression of the \( \lambda_1 \) light chain in \( r_\lambda^+ \) 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 \( \lambda_1 \). As examples, a reversion in a mutant regulatory element would not be translated during \( \lambda_1 \) light chain synthesis. A reversion in a nonsense codon in that region of DNA encoding the \( \lambda_1 \) amino-terminal precursor piece would be translated but not detected because it is cleaved from the \( \lambda_1 \) light chain (8).

We can approximate (9) what the mutation rate must be to generate the steady-state number of \( \lambda_1 \)-bearing ASCs enumerated by fluorescent anti-\( \lambda_1 \) cell surface staining of \( r_\lambda^+ \) homozygote splenic lymphocytes. If \( N \) is the number of rapid-dividing, short-lived cells which generate the immune system, \( \mu \) 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

\[
dM/dt = 0.69 a \mu N
\]

The mature immune system of a \( r_\lambda^+ \) homozygote there are \( \sim 10^4 \lambda_1 \) ASCs (Table V), and the immune system appears in \( t \sim 20 \) days, so

\[
dM/dt \sim 5 \times 10^2 \text{ revertants/day}
\]

\( N \) is taken to be on the order of \( 10^7 \) cells, since the steady-state adult B lymphocyte population is no larger than \( 2 \times 10^8 \) cells. Thus, \( a \) the mutation rate/base pair/division would have to be approximately \( 2.5 \times 10^{-5} \).

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

*A Summary of the Effect of the rX1+ and rx1lo Alleles on the λ1 ASC Population and on the Expression of λ1 Immunoglobulin*

| Inbred strain | Genotype | Total λ1 ASCs/ spleen* | νλ ASCs/ spleen | λ1ASCs | Relative ratio of λ1ASCs (S/JL = 1) | λ1 lg (total Ig) | Relative ratio of λ1-lg to light chain (S/JL = 1) |
|---------------|----------|------------------------|----------------|--------|---------------------------------|----------------|-----------------------------------------------|
| BALB/c        | rX1 +    | 2.5 × 10^5             | 1.4            | 52     | 1.5                             | 54             |                                               |
| (BALB/c × SJL)F1 | rX1 +/rX1lo | 10^4                    | —              | —      | 21§                            | —              |                                               |
| SJL           | rX1lo/rX1lo | 10^4                    | —              | 0.027  | 1                               | 0.028          | 1                                             |
| SJLαα         | rX1lo/rX1lo | 10^4                    | —              | —      | —                              | —              |                                               |

* Calculated from Table IV, assuming 5 × 10^3 ASCs/spleen.
§ Based on the estimate of 1 v<sub>λ</sub> gene/10<sup>2</sup> total ν<sub>λ</sub> gene (18).
§§ Calculated from Table IV, assuming that all ASCs stain with one of the FITC-conjugated κ- or λ<sub>λ</sub> antibodies. (BALB/c × SJL)F1 are assumed to have the same number of κ-ASCs as BALB/c.
|| Data from Table I. (BALB/c × SJL)F1 are assumed to have the same amount of κ-lg in normal serum as BALB/c.
|---|---|---|---|---|---|---|---|

randomly in each ASC. The result would be a gene dosage effect because the stem cell expressing the rX1lo allele would have a low probability of becoming a functional λ1 ASC while the stem cell expressing the rX1+ allele would have a high probability of becoming a functional λ1 ASC.

**What Regulatory Function Might the rX1 Locus Encode?** An acceptable model must account for the finding that, in rX1lo homozygotes, the rX1lo allele greatly reduces the number of λ1 ASCs but the few λ1 ASCs present express a functional λ1 light chain indistinguishable from wild-type. In one such molecular level model the rX1lo allele represents a single base change in a DNA palindrome involved in the translocation event which makes the 5'-end of the ν<sub>λ</sub> gene and the 3'-end of the intron-C<sub>λ</sub> 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, rX1 +/rX1lo, which is undergoing differentiation to a B cell expressing the λ1 light chain. Initially the stem cell undergoes allelic exclusion and translocation of ν<sub>λ</sub> to intron-C<sub>λ</sub>. If the rX1+ allele is expressed the palindromic sequences are nicked with 100% probability and successful translocation occurs. If the rX1lo allele is expressed, endonuclease nicking and translocation is successful with only 2% probability. The unsuccessful cells committed to λ1 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 λ1 in rX1+ /rX1+, rX1+ /rX1lo, and rX1lo/rX1lo mice should be 100:51:2, respectively, and that is the case (Table V). Once a cell has undergone successful translocation and expresses λ1, whether it is rX1lo or rX1+, it functions equally well.

In this regard, it should be noted that although the λ1 and λ2 light chain class are probably each coded for by one ν and one c gene, the λ1:λ2 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 λ1 and λ2. Such a model would predict that the 5'-ν or the 3'-intron-c palindromes are different for λ1 and λ2, but they use the same endonuclease. We are aware of alternative explanations. For example, it may be that λ1 and λ2 are translocated at the same frequency and expressed with a different average family of v<sub>ν</sub> in ASCs, and that the expression of λ1 with its v<sub>ν</sub> repertoire...
may produce more antigen-binding specificities than the corresponding $\lambda_{2VH}$ combinations. In that case the 4:1 ratio of $\lambda_1/\lambda_2$ in normal Ig would reflect a preferential antigenic selection for $\lambda_1VH$ combinations.

The Regulation of a Specific Antibody Response in the $\lambda_1$ Class by the $\tau_{\lambda_1}$ Locus. In animals possessing the responder heavy chain locus ($\lambda_1V\alpha_{13}^{3}$), the $\tau_{\lambda_1}v_{\lambda_1}$ homozygotes shows wide fluctuations in the magnitude of the anti-\(\alpha(1,3)\) dextran response, while the $\tau_{\lambda_1}+\$ homozygotes shows a uniform, high $\lambda_1$ anti-\(\alpha(1,3)\) dextran response. Since the effect of the $\tau_{\lambda_1}v_{\lambda_1}$ allele is to reduce the number of $\lambda_1$-bearing ASCs, it is reasonable to assume that the variability in the response of $\tau_{\lambda_1}v_{\lambda_1}$ homozygotes is due to a large variance in the distribution of ASCs expressing $\lambda_1V\alpha_{13}^{3}$ per individual. Since it was found that the $\tau_{\lambda_1}v_{\lambda_1}$ allele reduces the number of $\lambda_1V\alpha_{13}^{3}$ 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 $\tau_{\lambda_1}v_{\lambda_1}$ homozygotes can be calculated from the fraction of the sample that fail to give a $\lambda_1V\alpha_{13}^{3}$ primary response to $\alpha(1,3)$ dextran (Fig. 3). The fraction of nonresponders, $r_0$, is 0.208. For $r_0$, $p(r_0) = e^{-m}$, 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 $\lambda_1V\alpha_{13}^{3}$ and correspond to one responding unit may be estimated by dividing the number of $\lambda_1V\alpha_{13}^{3}$ ASCs in $\tau_{\lambda_1}v_{\lambda_1}$ homozygotes

### Table VI

The Distribution in the Magnitude of the Anti-\(\alpha(1,3)\) Response in the $\lambda_1$ Class of $\tau_{\lambda_1}v_{\lambda_1}$ Homozygotes Fits a Poisson Distribution

| $r$ | Fraction of sample | Predicted distribution for an average number of responding units, $m = 1.6$ | Observed distribution for the magnitude of $\lambda_1V\alpha_{13}^{3}$ responsiveness* |
|-----|--------------------|-----------------------------------------------|-----------------------------------------------|
| 0   | 0.20               | 0.21 (5/24)                                   | 0 (<25)                                       |
| 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.
(Table V) by the average number of responding units. Hence, one responding unit corresponds to \((10^6 + 1.6) \approx 50\) ASCs. Since the total number of \(\lambda_1\) ASCs is 50-fold higher in wild-type \(\lambda_{+}\) homozygotes than in \(\lambda_{lo}\) (Table V), both the number of responding units and also the number of ASCs for \(v_{Hl}\) should be increased 50-fold. Thus, for the wild-type, \(m = (1.6 \times 50) \approx 80\) responding units and there are \((80\) responding units \(\times 50\) ASCs/responding unit) \(\approx 4 \times 10^7\) ASCs expressing \(v_{Hl}\).

In fact, over 100 \(\lambda_{+}\) individuals have been immunized with \(\alpha(1,3)\) dextran (Fig. 3, partial data shown) and no nonresponders have been found.

If the number of wild-type \(v_{Hl}\) ASCs is typical of any particular germ-line combination and there are about \(10^6\) \(v_{L}\) germ-like combinations (15), then the total number of ASCs per individual would be \((4 \times 10^6) (10^6) \approx 4 \times 10^7\). Of \(4 \times 10^7\) ASCs with germ-line encoded \(v_{L}\) only about \(4 \times 10^5\) 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 \(\lambda_1\) Class by the \(\lambda_1\) Locus. Another phenotypic effect of the \(\lambda_1\) locus which must be accounted for in terms of different numbers of \(\lambda_1\)-bearing ASCs in the regulation of the level of \(\lambda_1\) associated with normal immunoglobulin in \(\lambda_{+}\) and \(\lambda_{lo}\) homozygotes. The analysis rests on two observations. The first observation is that for \(\lambda_{+}\) homozygotes the ratio of \(\lambda_1:\lambda_2\) is the same as the ratio of \(\lambda_1:v_\alpha\) genes.

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

The ratio of \(\lambda_1/v_{\alpha}\) in virgin ASCs represents the probability of expression of the \(v_{\alpha}\) gene relative to the total \(v_{L}\) pool. Since this ratio is the same as the ratio of \(v_{\alpha}\) germ-line genes, the probability of expression of each \(v_{\alpha}\) gene is, on average, the same. This means that the \(\lambda_{+}\) regulatory gene operates with the same efficiency as an average \(\lambda_{+}\) regulatory gene. The ratio of \(\lambda_1/v_{\alpha}\) in serum Ig represents the probability of induction of a given \(\lambda_1\) ASC relative to a \(\kappa\)-ASC. Since this ratio is the same as the ratio of \(\lambda_1/v_{\alpha}\) 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 \(\lambda_{lo}\) mutation (as well as the \(\lambda_{lo}\)-like behavior of the \(\lambda_2\) 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 \(v_{\alpha}\) genes.

The gene dosage expression of \(\lambda_1\) poses a paradox, for it is not immediately obvious that the level of serum \(\lambda_1\) Ig should depend strictly on the number of ASCs initially expressing \(\lambda_1\). The ratio of \(\lambda_1\)-bearing ASCs in \(\lambda_{lo}/\lambda_{lo}:\lambda_{lo}/\lambda_{+}\) genotypes is the same as the ratio of \(\lambda_1\) light chains associated with normal immunoglobulin in each genotype, and is approximately 1:25:50. Clearly, the \(v_{Hl}\) repertoire in a \(\lambda_{+}\) homozygote and a \(\lambda_{lo}/\lambda_{+}\) 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 VL 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 \( r_{\lambda,0} \) homozygotes, ASCs expressing various \( v_{\lambda, V_H} \) combinations replace the lost ASCs which would have expressed \( v_{\lambda, V_H} \) combinations. These \( v_{\lambda, V_H} \) ASC replacements become inducible by the determinants in the antigenic universe which preferentially would have induced their missing \( v_{\lambda, V_H} \) counterparts. This conclusion is supported by a study (17) with the \( \kappa \)-locus in rabbits. Suppression of the expression of \( \kappa \) 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 \( \lambda \)).

Is the Regulatory Gene Marked by the \( r_{\lambda,0} \) Allele an Example of an Element Controlling the Expression of an Entire Light Chain (Translocon) or of a Single \( v \)-Gene? Since the \( \lambda_1 \) translocon consists of only one \( v \)-gene, this question is unresolved. Given that the ratio of \( \lambda_1/\kappa \) ASC, serum Ig, and germ-line \( v \)-genes is \( \sim 1/100 \), two modes of expression of \( v \)-genes are reasonable. If the \( r_{\lambda_1} \) gene regulates expression of the \( \lambda_1 \) translocon then the stem cell first decides which translocon to activate, \( \lambda_1 \) or \( \kappa \), after which the activated translocon expresses sequentially all of its \( v \)-genes. Such a mode of expression would make the ratio of \( \lambda_1/\kappa \) ASCs equal the \( v_{\lambda_1}/v_\kappa \) 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 \( r_{\lambda_1} \) allele could act to reduce the probability that the \( \lambda_1 \) translocon was chosen for activation. The \( bas \) mutation in rabbits (18), which results in the specific loss of the \( \kappa \)-light-chain class, might represent a defect in translocon expression.

Alternatively, if the \( r_{\lambda_1} \) gene regulates expression of a unique \( v \)-gene then the stem cell could simply activate \( v \)-genes randomly, treating \( v_{\lambda_1} \) and \( v_\kappa \) indifferentially. This latter mode of expression also would make the \( \lambda_1/\kappa \) ratio in ASCs equal to the \( v_{\lambda_1}/v_\kappa \) germ-line gene ratio. The model for the action of the \( r_{\lambda_1} \) 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 \( r_{\lambda_1} \) allele, it is probable that both types of regulatory genes operate and that we have revealed by the \( r_{\lambda_1} \) allele only one of them.

Do Regulatory Genes of the \( r_{\lambda_1} \)-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 \( \lambda_1 \) (this paper), rabbit-\( \kappa \) (17) and murine \( \gamma_G_{2a} \) (19). If there is a compensatory increase it appears to come from the other classes, \( \kappa \) for murine \( \lambda_1 \), \( \lambda \) for rabbit \( \kappa \) and an unknown \( \gamma_G \) class for murine \( \gamma_G_{2a} \). 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 \(\text{cH}\)-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 \([\text{IgM} + \text{IgD}]\) 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 \(\text{cis}\) 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 \(r_\lambda\) type.

Summary

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

In the homozygous state, the present of the \(r_\lambda,lo\) allele results in a 50-fold reduction in the number of \(\lambda_1\) antigen-sensitive, bone-marrow derived lymphocytes (ASCs) compared to the presence of the \(r_\lambda,+\) allele. However, those few \(\lambda_1\)-ASCs present in \(r_\lambda,lo\) homozygotes can be induced normally to produce \(\lambda_1\) light chains indistinguishable from those found in \(r_\lambda,+\) homozygotes. The reduction in \(\lambda_1\)-ASC's due to the \(r_\lambda,lo\) allele results both in a reduction in the amount of \(\lambda_1\) Ig in the serum and also in a large variation in the magnitude of the \(\lambda_1\) 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 \(\lambda_1\) light chain expression \((r_\lambda\) locus) shows a clear gene dosage effect with \(r_\lambda,lo/r_\lambda,lo\) heterozygotes having \(\frac{1}{2}\) the number of \(\lambda_1\)-ASCs and \(\frac{1}{2}\) the amount of serum \(\lambda_1\) Ig as \(r_\lambda,+/r_\lambda,+\) homozygotes. This fact permits an analysis of the relationship between germ-line v-genes and their individual expression in serum Ig.

The \(r_\lambda\) locus controls specifically a DNA-level event which occurs in stem cells as they become committed to \(\lambda_1\) light chain expression. We postulate that the \(r_\lambda\) locus represents one of the DNA level recognition sites involved in the translocation event which places the \(v_\lambda\) and \(c_\lambda\) structural genes in a transcriptional unit.

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