Brief Definitive Report

INDUCTION OF λ_1-IMMUNOGLOBULIN IS DETERMINED BY A REGULATORY GENE ($r_{\lambda 1}$) LINKED (OR IDENTICAL) TO THE STRUCTURAL ($c_{\lambda 1}$) GENE

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The expression of the λ_1-immunoglobulin light chain has been attributed to a gene, $r_{\lambda 1}$, which acts *in cis* to the structural gene, and for which alleles exist that produce different λ_1-phenotypes (1). We demonstrate here that the $r_{\lambda 1}$-locus is tightly linked to (or identical with) the structural gene $c_{\lambda 1}$, which encodes the constant region of λ_1 light chains. This finding raises fundamental questions about regulation of the expression of the gene loci encoding the light (L) and heavy (H) chain immunoglobulin subunits.

Mice exhibiting the $r^{lo}_{\lambda 1}$-phenotype (e.g., SJL, SJA) express a serum level of λ_1Ig at least 30 times lower than that observed in $r^{+}_{\lambda 1}$ strains (e.g., BALB/c). Heterozygotes express a level of serum λ_1-Ig intermediate between that of the parents, thus demonstrating a gene dosage effect. Moreover, although mice of the $r^{lo}_{\lambda 1}$-phenotype, respond to the immunogen α(1,3)dextran (B1355) uniquely in the λ_1 class, they respond in a "patchy" manner, whereas mice expressing $r^{+}_{\lambda 1}$ respond uniformly to this antigen, yielding a high titer antibody. These data suggest that the $r_{\lambda 1}$ locus controls either the number of virgin B cells expressing λ_1-Ig or their inducibility, and the $r_{\lambda 1}$ locus is expressed *in cis* to the structural λ_1-gene (1).

The structural λ-genes map on chromosome 16 of the mouse (2). Their order is not firmly established (3), but the $\lambda_{2,4}$ cluster is thought to lie 5′ to the $\lambda_{3,1}$ cluster (4, 5). The recent availability of $c_{\lambda 1}$ gene markers (reference 6, and B. Blomberg, personal communication), as well as a family of BALB/cKe(C) × SJL/J(J) recombinant inbred strains has enabled us to address the question of the linkage between the structural ($c_{\lambda 1}$) and regulatory ($r_{\lambda 1}$) loci.

A polymorphic marker in the constant region of the structural gene for λ_1 has been identified both at the protein (7, 8) and DNA level (reference 6, and B. Blomberg, personal communication).

A comparison of the DNA sequences of BALB/c and SJL/J λ_1 structural genes revealed only one difference, which is in the constant region (reference 6, and B. Blomberg, personal communication) and corresponds to the allotype difference observed at the protein level. In the SJL/J mouse, a G → T transversion in codon 155 (GGT) of $C_{\lambda 1}$ results in a replacement of the glycine present in BALB/c λ_1 by a valine (GTT). The consequence of this mutation is the loss in SJL/J of a cleavage site present in BALB/c for the restriction endonuclease KpnI. No further differences

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between these two strains could be detected by heteroduplex analysis (6). The absence of any sequence differences 5′ to $v_{\lambda 1}$ implies that altered λ_1 levels are not due to changes in transcription arising from a structural modification in the promoter region. Also identical are the fusion sequences 3′ to $v_{\lambda 1}$ and 5′ to $j_{\lambda 1}$, and the RNA processing recognition sequences for the two introns $l_{\lambda 1}$-$v_{\lambda 1}$ and $j_{\lambda 1}$-$c_{\lambda 1}$. That the $r\lambda_1^{lo}$-phenotype arises from either inversions of $v_{\lambda 1}$, $j_{\lambda 1}$, and $c_{\lambda 1}$ or from large insertions or deletions also appears to be excluded.

It should be stressed that the gly/val interchange does not affect detectably the behavior of the corresponding λ_1-Ig secreted in serum. The specificity (apparent affinity), idiotype, complement fixation, and stability characteristics of BALB/c and SJA λ_1-Ig are indistinguishable (1).

The principle of our mapping is the following: we have used a monoclonal rat-anti-mouse λ_1 to assay the λ_1-Ig levels in the serum, and from this infer which $r_{\lambda 1}$ allele is expressed by each strain of the [BALB/cKe(C) × SJL(J)] recombinant inbred family. The $c_{\lambda 1}$ allele expressed by each strain was determined in two ways: (*a*) serum from each recombinant inbred was allotyped, and (*b*) liver DNA from each strain was tested for the presence of the KpnI cleavage site via the method of Southern (9).

Materials and Methods

Mice. BALB/cKe, SJL/J (the latter originally obtained from The Jackson Laboratory, Bar Harbor, ME), and SJA mice (a gift from Dr. Roy Riblet) were bred at the Salk Institute. Recombinant inbred mice were constructed by standard methods (10) (Table I).

Identification of Restriction Fragments. 40 μg of liver DNA, prepared by procedure B described by Cory et al. (11), was fractionated by horizontal agarose gel electrophoresis. DNA was blotted according to the method of Southern (9) as modified by Alwine (12) and hybridized (13) to a purified (14) $c\lambda_1$ probe (a gift of Dr. Bonnie Blomberg).

Antisera. Culture supernatants from hybridomas Ls136 [SJL anti-λ_1 (16)] and L22.18.2 [rat anti-λ_1 (17)] were cut with 45% ammonium sulfate and purified over Sepharose-4B protein A columns.

Rabbit antisera (anti-λ_1, anti-κ, anti-μ, and anti-γ) and myeloma proteins used for standards [J558($\lambda_1\alpha$), MOPC104E($\lambda_1\mu$), Y5781($\kappa\mu$)] were purified as described previously (15).

Enzyme-linked Immunosorbent Assay (ELISA). Polyvinyl plates (Cooke, 1-220-25), coated with 0.5 μg of Ls136/7 (16) or 0.25 μg L22.18.2 (17), were incubated overnight at 4°C. After unreacted sites were blocked by incubating plates 1 h with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), sera and standard proteins (250 to 0.5 ng) were added to the wells and incubated for 2 h at 37°C and washed with PBS. Alkaline phosphatase-conjugated antibodies (anti-λ or anti-κ) (18) diluted in PBS containing 1% BSA were added, and after overnight incubation at 4°C, followed by washing with PBS, 150 μg of *p*-nitrophenyl phosphate (Merck Chemical Div., Merck & Co., Inc., Rahway, NJ) was added to each well. The optical density was measured at 412 nm with a Titertek Multiskan Photometer 45 min later. Total immunoglobulin was measured as above with plates initially coated with rabbit anti-μ or γ.

Results

The Expression of λ_1 *in [BALB/cKe(C) × SJL/J(J)]*F_1 *Mice.* Quantitation of the λ_1Ig present in the serum of F_1 mice (with L22.18.2 or Ls136) confirms that their λ_1-Ig level is intermediate between BALB/cKe and SJL/J mice (1). All of the λ_1-Ig is of the BALB/c type, i.e., $r_{\lambda 1}^{+}$ and $r_{\lambda 1}^{lo}$ seem to be each expressed *in cis* to their respective structural $c_{\lambda 1}$ genes.

Linkage of Structural and Regulatory λ_1 *Loci in (C × J) Mice.* The total λ_1-level in serum from each recombinant inbred (RI) strain was assayed using L22.18.2 rat anti-λ_1 (Table I). The identification of the structural gene present in each RI was assayed

TABLE I

Structural and Regulatory Gene Expression

Strain	$r_{\lambda 1}$-Allele (assayed with L22.18.2)	$c_{\lambda 1}$-Allele determined		H-2	Igh
		with Ls136	by presence of KpnI Site		
BALB/cKe	C	C	C	d	a
SJL/J	J	J	J	s	b
(BALB/cKexSJL/J)F_1	C/J	C/J	C/J	d/s	a/b
SJA	J	J	J	s	a
C × J 1	C	C	C	d	a
C × J 3	J	J	J	d	b
C × J 4	C	C	C	s	a
C × J 6	J	J	J	s	a
C × J 8	C	C	C	d	b
C × J 9	J	J	J	d	b
C × J 10	J	J	J	d	b
C × J 11	J	J	J	d	b
C × J 13	J	J	J	s	b
C × J 15	J	J	J	s	b

C, BALB/c allele. J, SJL/J allele. C/J, the (BALB/cKexSJL/J)F_1 showed an intermediate level of λ_1-Ig in serum (assayed with L22.18.2) all of which was of the BALB/c λ_1^+ type (assayed with Ls136) (see text). Calculation of linkage (19): During the successive generations of inbreeding in the preparation of an RI line, there are multiple opportunities for recombination between linked loci. The probability of fixing a recombinant genotype (R) is $4r/(1 + 6r)$ where r is the probability of recombination in a single meiosis. Since $r = R/(4-6R)$ and $R < 0.1$, $r < 0.029 \pm 0.008$. The $r_{\lambda 1}$-allele was inferred from the phenotype determined by an ELISA using monoclonal L22.18.2 rat anti-mouse λ_1. Mice with <5 μg/ml λ_1-Ig were designated $r_{\lambda 1}^{lo}$, while those above 30 μg/ml were scored $r_{\lambda 1}^+$. The λ_1 polymorphic marker was determined with monoclonal Ls136. The presence of the KpnI site was detected by Southern blot analysis.

using Ls136, SJL anti-λ_1. Only (C × J) RI 1, 4, and 8 demonstrated total levels of λ_1 comparable to that expressed by BALB/c mice. All others were similar to SJL in their low λ_1 level. All exhibited normal κ levels. Only those RI strains exhibiting the BALB/c phenotype reacted with Ls136. Southern blot analysis confirmed that the BALB/c structural gene was present in these strains (Fig. 1). The DNA from (C × J)RI 1, 4, and 8 were of the BALB/c type exhibiting a 4.1–4.2 kb BALB/c band due to the presence of the KpnI cleavage site. The DNA from the other (C × J) RI strains were of the SJL type exhibiting a band of size 7.9–8.1 kb due to the absence of this site. Therefore the structural gene $c_{\lambda 1}$ and the regulatory gene $r_{\lambda 1}$ are linked within 2.9 map units (19).

Responsiveness of λ_1^{lo}-SJA to α(1,3)-Dextran is Not Due to a Somatic Mutation. Since SJA is capable of mounting a patchy λ_1 response to α(1,3)dextran (B1355), analysis of this antibody with Ls136 should test directly whether a somatic reversion in the $C_{\lambda 1}$ region accounts for its expression. If such a mutation (val → gly) permitted λ_1 expression, the antibody induced should express the polymorphic marker observed in $r_{\lambda 1}^+$ strains. Analysis of the anti-dextran produced by both SJA and RI strain 6 (also Igh^a and $r_{\lambda 1}^{lo}$) showed the absence of reversion (Table II).

Discussion

The expression *in cis* and the tight linkage between the regulatory and structural loci permits consideration of only two types of explanation for the λ_1^{lo}-phenotype. (*a*)

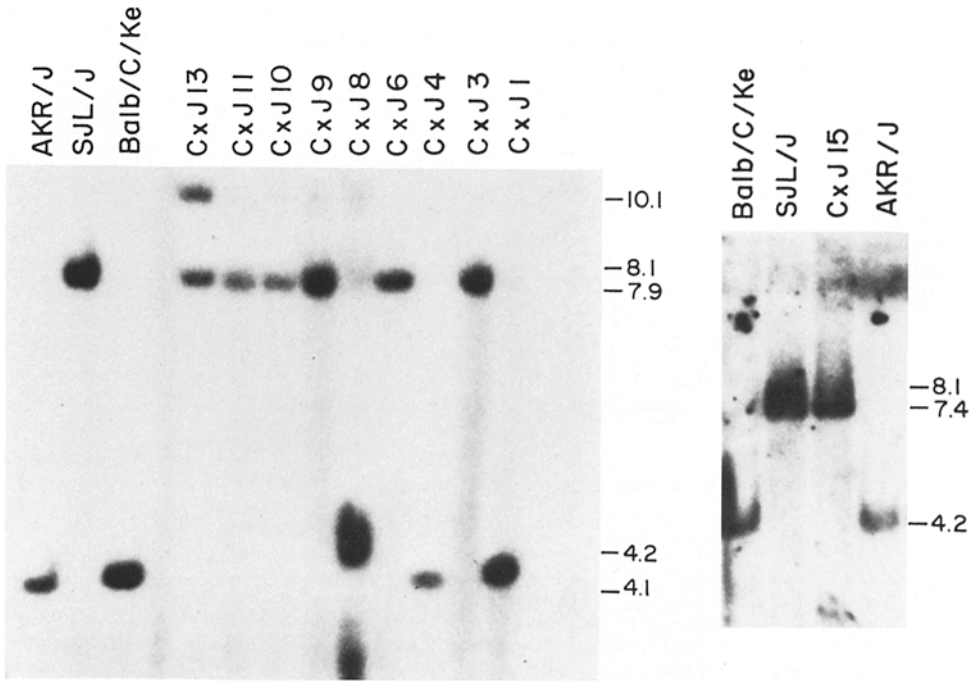

FIG. 1. Southern blot analysis of $c_{\lambda 1}$-EXON for presence of polymorphic KpnI cleavage site.

The expression of λ_1-Ig is determined by the G → T interchange in codon 155 of the $c_{\lambda 1}$-gene. (*b*) The expression of λ_1-Ig is determined by a *cis* acting gene, $r_{\lambda 1}$, linked to (but distinct from) the $c_{\lambda 1}$-gene.

If the mutation G → T is responsible for the λ_1^{lo}-phenotype, then the corresponding glycine → valine replacement at position 155 must affect the number of adult λ_1 expressing B cells capable of being induced (1). This could occur in two ways: (*a*) The formation of a complex between light and heavy chains has been postulated to stop further rearrangements in the differentiating B cell, resulting in the stable expression of Ig loci (Claverie, J.-M., and R. Langman, manuscript in preparation). A "stopless" state might arise when a gly/val interchange occurs, rendering this complex incapable of preventing further rearrangements at other loci. The consequence would be a reduction in the number of virgin λ_1 expressing B cells. Those B cells that encounter antigen in the fleeting period during which they express λ_1-Ig would be induced to a stable memory state. This would account for the patchy response of SJA to dextran. (*b*) Alternatively, the alteration in the structure of the λ_1-light chain could affect the signaling interaction of B cells with antigen, thus lowering the efficiency of induction. This too would lead to a patchy response. If the virgin B cell is more difficult to induce, then the triggering event must be mediated by a conformational change in the Ig receptor upon interaction with antigen, an interpretation that is in contrast to the generally held view that the triggering event arises from the aggregation of Ig receptors by antigen. This "inductionless" model is favored by the finding that newborn BALB/c (λ_1^+) and SJL/J (λ_1^{lo}) mice have equal numbers of λ_1-bearing B cells, but the number of BALB/c λ_1-bearing B cells increases while the SJL/J λ_1-bearing B cells decreases as the animals age (8).

TABLE II

The λ_1 Antibody Response in $r_{\lambda 1}^{lo}$ Strains is Not Due to Somatic Reversion at $C_{\lambda 1}$ (Val) to Wild Type (Gly)

Strain	$r_{\lambda 1}$-allele	Igh	Reactivity of λ_1 anti-α(1,3)-dextran with	
			Ls136	L.22.18.2
BALB/c/Ke	+	a	+	+
SJA	Lo	a	−	+
C × J6	Lo	a	−	+

The assay for the presence of the polymorphic constant region marker was performed by ELISA using monoclonal antibodies Ls136 and L22.18.2. The primary anti-α(1,3)dextran response was determined on day 8 after immunization with 100 μg of B1355 dextran in Freund's complete adjuvant. All levels of λ_1 anti-α(1,3)dextran were ~65 μg/ml. The κ levels were equivalent in all mice.

If the λ_1^{lo}-phenotype is due to a closely linked *cis*-acting regulatory sequence or gene ($r_{\lambda 1}$), the λ_1-Ig receptor once expressed would behave identically during induction in both BALB/c and SJA mice. The patchy response would again be explained by a insufficiency of λ_1-bearing B cells. This might be the consequence of a mutation resulting in an altered DNA conformation or in a modification of a regulatory sequence by methylation. Since B cells in SJL and BALB/c newborns appear to express λ_1 at similar levels while adults are widely different (8), any alteration in conformation or methylation would have to be expressed as the animal matures.

While a regulatory alteration might affect both λ_1 and λ_2, the $c_{\lambda 1}$ alteration is expected to affect only λ_1, with the proviso that if there were a failure to terminate rearrangements an increase in λ_2-bearing B cells might occur. λ_2 levels in the serum of adult SJL/J have been shown to be lower than are those in adult BALB/c (8, 20), and these observations led to the suggestion that the λ-phenotypes are due to T cell regulatory effects, "suppressive" or "helper" (8, 21). However, an effect on λ_2 of the $r_{\lambda 1}^{lo}$-gene is questionable. The effect the $r_{\lambda 1}^{lo}$ on λ_2 is an order of magnitude less than that observed for λ_1, and there is no correlation in various mouse strains between λ_2 levels and the allele of $r_{\lambda 1}$, lo or +, which is expressed. Further, this suggestion does not explain why the specific control of λ_1 expression by T cells should be linked to the structural $c_{\lambda 1}$-gene.

Summary

The *cis*-acting gene regulating specifically the inducibility of λ_1-bearing B cells has been mapped within 2.9 cM of the structural gene. If the λ_1^{lo}-phenotype is due to the gly → val interchange in $c_{\lambda 1}$, then an argument can be made that (*a*) the λ_1^{lo}-phenotype is due to inefficient induction of λ_1^{lo}-bearing B cells and (*b*) B cell triggering is dependent upon a conformational change in the Ig receptor upon interaction with antigen. If the λ_1^{lo}-phenotype is due to a regulatory sequence linked to the structural $c_{\lambda 1}$-gene, then it must control the expression of the λ_1-locus during development into adulthood, e.g., by an effect on methylation.

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