

INDUCTION OF  $\lambda_1$ -IMMUNOGLOBULIN IS DETERMINED BY A  
REGULATORY GENE ( $r_{\lambda_1}$ ) LINKED (OR IDENTICAL) TO THE  
STRUCTURAL ( $c_{\lambda_1}$ ) GENE

BY RUTH EPSTEIN,\* KATHERIN LEHMANN,‡ AND MELVIN COHN‡

*From the Developmental Biology Laboratory, Salk Institute, San Diego, California 92138*

The expression of the  $\lambda_1$ -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  $\lambda_1$ -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  $\lambda_1$  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  $r_{\lambda_1}^0$ -phenotype (e.g., SJL, SJA) express a serum level of  $\lambda_1$ Ig at least 30 times lower than that observed in  $r_{\lambda_1}^+$  strains (e.g., BALB/c). Heterozygotes express a level of serum  $\lambda_1$ -Ig intermediate between that of the parents, thus demonstrating a gene dosage effect. Moreover, although mice of the  $r_{\lambda_1}^0$ -phenotype, respond to the immunogen  $\alpha(1,3)$ dextran (B1355) uniquely in the  $\lambda_1$  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  $\lambda_1$ -Ig or their inducibility, and the  $r_{\lambda_1}$  locus is expressed *in cis* to the structural  $\lambda_1$ -gene (1).

The structural  $\lambda$ -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)  $\times$  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  $\lambda_1$  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  $\lambda_1$  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  $\rightarrow$  T transversion in codon 155 (GGT) of  $C_{\lambda_1}$  results in a replacement of the glycine present in BALB/c  $\lambda_1$  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

\* Supported by grant CA-09174 from the National Institutes of Health.

‡ Supported by grant A-10575 from the National Institute of Allergy and Infectious Diseases.

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  $\lambda_1$  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  $i_{\lambda 1-v_{\lambda 1}}$  and  $i_{\lambda 1-c_{\lambda 1}}$ . That the  $r_{\lambda 1}^{10}$ -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  $\lambda_1$ -Ig secreted in serum. The specificity (apparent affinity), idiotype, complement fixation, and stability characteristics of BALB/c and SJA  $\lambda_1$ -Ig are indistinguishable (1).

The principle of our mapping is the following: we have used a monoclonal rat-anti-mouse  $\lambda_1$  to assay the  $\lambda_1$ -Ig levels in the serum, and from this infer which  $r_{\lambda 1}$  allele is expressed by each strain of the [BALB/cKe(C)  $\times$  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  $\mu$ g 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- $\lambda_1$  (16)] and L22.18.2 [rat anti- $\lambda_1$  (17)] were cut with 45% ammonium sulfate and purified over Sepharose-4B protein A columns.

Rabbit antisera (anti- $\lambda_1$ , anti- $\kappa$ , anti- $\mu$ , and anti- $\gamma$ ) 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  $\mu$ g of Ls136/7 (16) or 0.25  $\mu$ g 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- $\lambda$  or anti- $\kappa$ ) (18) diluted in PBS containing 1% BSA were added, and after overnight incubation at 4°C, followed by washing with PBS, 150  $\mu$ g 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- $\mu$  or  $\gamma$ .

### Results

*The Expression of  $\lambda_1$  in [BALB/cKe(C)  $\times$  SJL/J(J)]F<sub>1</sub> Mice.* Quantitation of the  $\lambda_1$ -Ig present in the serum of F<sub>1</sub> mice (with L22.18.2 or Ls136) confirms that their  $\lambda_1$ -Ig level is intermediate between BALB/cKe and SJL/J mice (1). All of the  $\lambda_1$ -Ig is of the BALB/c type, i.e.,  $r_{\lambda 1}^+$  and  $r_{\lambda 1}^0$  seem to be each expressed *in cis* to their respective structural  $c_{\lambda 1}$  genes.

*Linkage of Structural and Regulatory  $\lambda_1$  Loci in (C  $\times$  J) Mice.* The total  $\lambda_1$ -level in serum from each recombinant inbred (RI) strain was assayed using L22.18.2 rat anti- $\lambda_1$  (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 $\times$ J 1	C	C	C	d	a
C $\times$ J 3	J	J	J	d	b
C $\times$ J 4	C	C	C	s	a
C $\times$ J 6	J	J	J	s	a
C $\times$ J 8	C	C	C	d	b
C $\times$ J 9	J	J	J	d	b
C $\times$ J 10	J	J	J	d	b
C $\times$ J 11	J	J	J	d	b
C $\times$ J 13	J	J	J	s	b
C $\times$ 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  $\lambda_1$ -Ig in serum (assayed with L22.18.2) all of which was of the BALB/c  $\lambda_1^+$  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  $\lambda_1$ . Mice with  $< 5 \mu\text{g/ml}$   $\lambda_1$ -Ig were designated  $r_{\lambda_1}^0$ , while those above  $30 \mu\text{g/ml}$  were scored  $r_{\lambda_1}^+$ . The  $\lambda_1$  polymorphic marker was determined with monoclonal Ls136. The presence of the KpnI site was detected by Southern blot analysis.

using Ls136, SJL anti- $\lambda_1$ . Only (C  $\times$  J) RI 1, 4, and 8 demonstrated total levels of  $\lambda_1$  comparable to that expressed by BALB/c mice. All others were similar to SJL in their low  $\lambda_1$  level. All exhibited normal  $\kappa$  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  $\times$  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  $\times$  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  $\lambda_1^{lo}$ -SJA to  $\alpha(1,3)$ -Dextran is Not Due to a Somatic Mutation.* Since SJA is capable of mounting a patchy  $\lambda_1$  response to  $\alpha(1,3)$ 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  $\rightarrow$  gly) permitted  $\lambda_1$  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<sup>a</sup> and  $r_{\lambda_1}^0$ ) 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  $\lambda_1^{lo}$ -phenotype. (a)

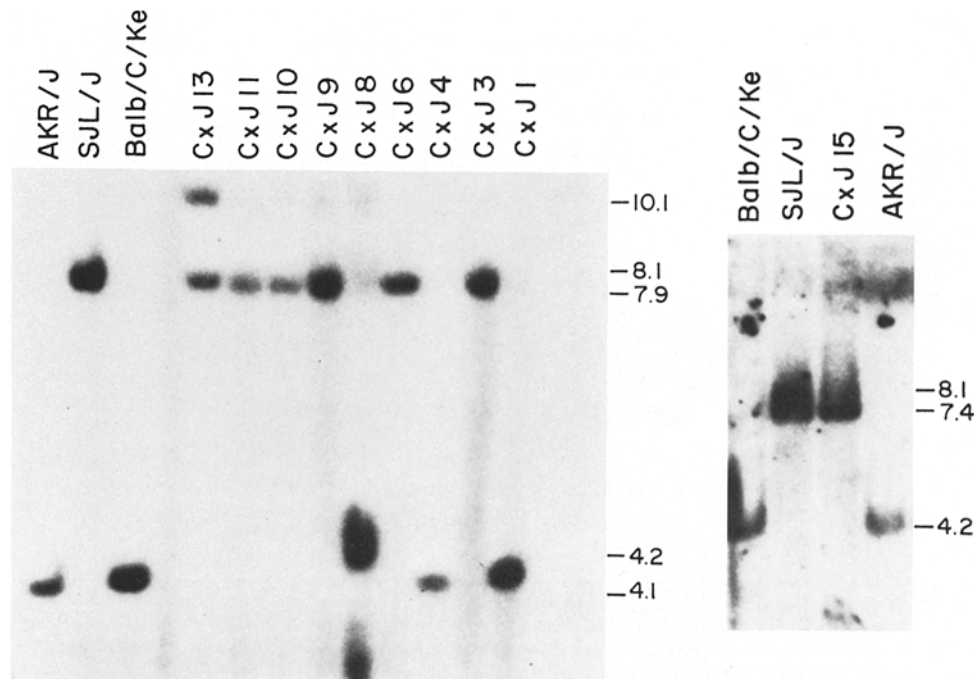


FIG. 1. Southern blot analysis of  $c_{\lambda 1}$ -EXON for presence of polymorphic KpnI cleavage site.

The expression of  $\lambda_1$ -Ig is determined by the G  $\rightarrow$  T interchange in codon 155 of the  $c_{\lambda 1}$ -gene. (b) The expression of  $\lambda_1$ -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  $\rightarrow$  T is responsible for the  $\lambda_1^{lo}$ -phenotype, then the corresponding glycine  $\rightarrow$  valine replacement at position 155 must affect the number of adult  $\lambda_1$  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  $\lambda_1$  expressing B cells. Those B cells that encounter antigen in the fleeting period during which they express  $\lambda_1$ -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  $\lambda_1$ -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 ( $\lambda_1^+$ ) and SJL/J ( $\lambda_1^{lo}$ ) mice have equal numbers of  $\lambda_1$ -bearing B cells, but the number of BALB/c  $\lambda_1$ -bearing B cells increases while the SJL/J  $\lambda_1$ -bearing B cells decreases as the animals age (8).

TABLE II  
*The  $\lambda_1$  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 $\lambda_1$ anti- $\alpha(1,3)$ -dextran with	
			Ls136	L.22.18.2
BALB/c/Ke	+	a	+	+
SJA	Lo	a	-	+
C $\times$ 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- $\alpha(1,3)$ dextran response was determined on day 8 after immunization with 100  $\mu$ g of B1355 dextran in Freund's complete adjuvant. All levels of  $\lambda_1$  anti- $\alpha(1,3)$ dextran were  $\sim 65$   $\mu$ g/ml. The  $\kappa$  levels were equivalent in all mice.

If the  $\lambda_1^{lo}$ -phenotype is due to a closely linked *cis*-acting regulatory sequence or gene ( $r_{\lambda_1}$ ), the  $\lambda_1$ -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  $\lambda_1$ -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  $\lambda_1$  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  $\lambda_1$  and  $\lambda_2$ , the  $c_{\lambda_1}$  alteration is expected to affect only  $\lambda_1$ , with the proviso that if there were a failure to terminate rearrangements an increase in  $\lambda_2$ -bearing B cells might occur.  $\lambda_2$  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  $\lambda$ -phenotypes are due to T cell regulatory effects, "suppressive" or "helper" (8, 21). However, an effect on  $\lambda_2$  of the  $r_{\lambda_1}^{lo}$ -gene is questionable. The effect the  $r_{\lambda_1}^{lo}$  on  $\lambda_2$  is an order of magnitude less than that observed for  $\lambda_1$ , and there is no correlation in various mouse strains between  $\lambda_2$  levels and the allele of  $r_{\lambda_1}$ , lo or +, which is expressed. Further, this suggestion does not explain why the specific control of  $\lambda_1$  expression by T cells should be linked to the structural  $c_{\lambda_1}$ -gene.

### Summary

The *cis*-acting gene regulating specifically the inducibility of  $\lambda_1$ -bearing B cells has been mapped within 2.9 cM of the structural gene. If the  $\lambda_1^{lo}$ -phenotype is due to the gly  $\rightarrow$  val interchange in  $c_{\lambda_1}$ , then an argument can be made that (a) the  $\lambda_1^{lo}$ -phenotype is due to inefficient induction of  $\lambda_1^{lo}$ -bearing B cells and (b) B cell triggering is dependent upon a conformational change in the Ig receptor upon interaction with antigen. If the  $\lambda_1^{lo}$ -phenotype is due to a regulatory sequence linked to the structural  $c_{\lambda_1}$ -gene, then it must control the expression of the  $\lambda_1$ -locus during development into adulthood, e.g., by an effect on methylation.

We thank Dr. Bonnie Blomberg for her generous assistance and advice and Dr. Rod Langman and Dr. Ed Palmer for their perceptive remarks.

Received for publication 28 December 1982 and in revised form 16 February 1983.

## References

1. Geckeler, W., J. Faversham, and M. Cohn. 1978. On a regulatory gene controlling the expression of the murine  $\lambda_1$  light chain. *J. Exp. Med.* **148**:1122.
2. D'Eustacio, P., D. Pravtchera, K. Marcu, and F. H. Ruddle. 1980. Chromosomal location of the structural gene cluster encoding murine immunoglobulin heavy chains. *J. Exp. Med.* **151**:1545.
3. Selsing, E., J. Miller, R. Wilson, and U. Storb. 1982. Evolution of mouse immunoglobulin  $\lambda$  genes. *Proc. Natl. Acad. Sci. USA.* **19**:4681.
4. Blomberg, B., A. Traunecker, H. Elsen, and S. Tonegawa. 1981. Organization of four mouse  $\lambda$  immunoglobulin genes. *Proc. Natl. Acad. Sci. USA.* **78**:3765.
5. Elliot, B. W., Jr., H. N. Eisen, and L. A. Steiner. 1982. Unusual association of V, J and C regions in a mouse immunoglobulin  $\lambda$  chain. *Nature (Lond.)*. **299**:559.
6. Arp, B., M. D. McMullan, and U. Storb. 1982. Sequences of immunoglobulin  $\lambda_1$  genes in a  $\lambda_1$  defective mouse strain. *Nature (Lond.)*. **298**:184.
7. Lieberman, R., W. Humphrey, Jr., and C. C. Chien. 1977. Identification and genetics of  $\lambda_1$  light-chain allotype in the mouse. *Immunogenetics.* **5**:515.
8. Takemori, T., and K. Rajewsky. 1981. Lambda chain expression at different stages of ontogeny in C57Bl/6, BALB/c and SJL mice. *Eur. J. Immunol.* **11**:618.
9. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503.
10. Bailey, D. W. 1971. Recombinant-inbred strains, an aid to finding identity, linkage, and function of histocompatibility and other genes. *Transplantation.* **11**:325.
11. Cory, S., and J. Adams. 1980. Deletions are associated with somatic rearrangement of immunoglobulin heavy chain genes. *Cell.* **19**:37.
12. Alwine, J. C., D. J. Kemp, and G. R. Stark. 1977. Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. *Proc. Natl. Acad. Sci. USA.* **74**:5350.
13. Wahl, G. M., M. Stern, and G. R. Stark. 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Acad. Sci. USA.* **16**:3683.
14. Brack, C., M. Hirama, R. Lenhard-Schuller, and S. Tonegawa. 1978. A complete immunoglobulin gene is created by somatic recombination. *Cell.* **15**:1.
15. Weiss, S., and K. Lehmann. 1981. The ratio of  $\kappa$ - to  $\lambda$ -bearing cells in murine fetal liver and bone marrow. In *The Immune System*. C. S. Steinberg, and Lefkovits, editors. Karger, Basel. **2**:33.
16. Reth, M., T. Imanishi-Kari, and K. Rajewsky. 1979. Analysis of the repertoire of anti-(4-hydroxy-3-nitro-phenyl) acetyl (NP) antibodies in the C56Bl/6 mice by cell fusion. II. Characterization of idiotopes by monoclonal anti-idiotope antibodies. *Eur. J. Immunol.* **9**:1004.
17. Weiss, S., K. Lehmann, and M. Cohn. 1983. Monoclonal antibodies to murine immunoglobulin isotypes. *Hybridoma.* **2**:49.
18. Kearney, F. J., A. Radbruch, B. Liesegang, and K. Rajewsky. 1979. A new myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. *J. Immunol.* **123**:1548.
19. Taylor, B. A. 1978. Recombinant inbred strains: use in gene mapping. In *Origins of Inbred Mice*. H. E. Morse, editor. Academic Press, New York. 423.
20. Cotner, T., and H. N. Eisen. 1978. The natural abundance of  $\lambda_2$ -light chains in inbred mice. *J. Exp. Med.* **148**:1388.
21. Reilly, E. B., A. R. Frankeldon, Jr., and H. N. Eisen. 1982. Synthesis of  $\lambda_1$ ,  $\lambda_2$  and  $\lambda_3$  light chains by mouse spleen B cells. *Eur. J. Immunol.* **12**:552.