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Immunology

Structure of a third murine immunoglobulin λ light chain variable region that is expressed in laboratory mice

(gene rearrangement)

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ABSTRACT **Recently, we reported evidence for the existence of an immunoglobulin λ light chain (λx) whose variable region differs from those encoded by the known V_λ gene segments $V_{\lambda 1}$ and $V_{\lambda 2}$. Expression of λx was detected in some hybridomas elicited by treatment of a BALB/c mouse with rabbit anti-λ2 antibodies coupled to bacterial lipopolysaccharide [Sanchez, P. & Cazenave, P.-A. (1987) *J. Exp. Med.* 166, 265–270]. We constructed a cDNA clone from one hybridoma (B6) that expresses the λx chain and determined the complete nucleotide sequence. The deduced amino acid sequence of $V_{\lambda x}$ is 30–33% identical with those encoded by $V_{\lambda 1}$ and $V_{\lambda 2}$ and by V_κ gene segments. The third hypervariable region of $V_{\lambda x}$ is four codons longer than those of the other murine variable gene segments. The expression of λx requires a genomic rearrangement that juxtaposes the $V_{\lambda x}$ gene with the $J_{\lambda 2}$–$C_{\lambda 2}$ joining–constant gene pair. Rabbit anti-$V_{\lambda x}$ antibodies detected the λx light chain in the normal sera of all laboratory mice tested. λx expression seems to be independent of λ1 expression, since both SJL and SJA strains, which are defective in λ1 production, express normal levels of λx chain.**

Two murine immunoglobulin light chain isotypes, κ and λ, have been described. The κ locus consists of 100–300 variable κ gene segments (V_κ), four functional joining segments (J_κ), and a single constant segment (C_κ). In contrast, the λ locus displays restricted heterogeneity. Three types of λ chains (called λ1, λ2, and λ3) are respectively encoded by the combination of $V_{\lambda 1}$–$J_{\lambda 1}$–$C_{\lambda 1}$, $V_{\lambda 2}$–$J_{\lambda 2}$–$C_{\lambda 2}$, and $V_{\lambda 1}$–$J_{\lambda 3}$–$C_{\lambda 3}$ gene segments (reviewed in refs. 1 and 2). Occasionally, $V_{\lambda 2}$ is found associated with either the $J_{\lambda 3}$–$C_{\lambda 3}$ or $J_{\lambda 1}$–$C_{\lambda 1}$ gene pairs (3–5). Two other constant gene segments ($C_{\lambda 4}$ and $C_{\lambda 5}$) have been described (1, 6). Whereas the $J_{\lambda 4}$–$C_{\lambda 4}$ gene pair was not found to be functional (7, 8), $C_{\lambda 5}$ coded for a putative non-antibody protein that was selectively expressed in pre-B cells (9, 10). Recently, we (11) demonstrated the existence of a distinct λ chain (which we call λx) expressed by hybridomas induced by treatment of a BALB/c mouse with rabbit anti-λ2 antibodies coupled to bacterial lipopolysaccharide (anti-λ2-LPS). Independently, Dildrop *et al.* (12) have discovered the same chain in the C57BL/6 strain. Here, we report the nucleotide sequence of the $V_{\lambda x}$ region,* which shows unexpected characteristics. $V_{\lambda x}$ exhibits only 33% amino acid identity with known V_λ segments and has an exceptionally long third hypervariable region [complementarity-determining region 3 (CDR3)]. The $V_{\lambda x}$ region undergoes rearrangement with the $J_{\lambda 2}$–$C_{\lambda 2}$ gene pair and is expressed in normal sera of laboratory mouse strains.

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MATERIALS AND METHODS

Cloning of $V_{\lambda x}$ cDNA. Poly(A)$^+$ mRNA from hybridoma B6 was fractionated by centrifugation in 5–20% sucrose gradients. The fractions containing light chain-encoding mRNA were analyzed by *in vitro* translation and pooled. cDNA was synthesized by the method of Gubler and Hoffman (13), using a cDNA synthesis kit (Amersham). cDNA was oligo(dC)-tailed and cloned into oligo(dG)-tailed *Pst* I site of Blue Scribe Plus vector (Vector Cloning Systems, Genofit, South Australia). A 200-base-pair (bp) *Ava* I–*Pst* I fragment derived from the clone pλII-I (14) was used as a $C_{\lambda 2}$ probe to screen the cDNA library (generously provided by T. J. Kindt, National Institutes of Health, Bethesda, MD).

Sequence Determination. The nucleotide sequences were determined, after subcloning into M13 phage vectors mp18 and mp19 (15), by the dideoxy chain-termination method (16).

Genomic Blot Analysis. DNA samples were isolated from SP2 cells, B6 hybridoma, or BALB/c liver. After digestion with appropriate restriction enzymes, 10-μg DNA samples were electrophoresed in a 0.8% agarose gel, blotted onto Hybond filters (Amersham), and hybridized with the $V_{\lambda x}$ probe depicted in Fig. 1*a*. Hybridization was carried out at 65°C in 450 mM NaCl/45 mM sodium citrate, pH 7. Final washes were with 15 mM NaCl/1.5 mM sodium citrate, pH 7, for 30 min at 65°C.

Immunochemical Analysis. All isotypic determinations were by specific inhibition assays (17). Isotype concentrations were deduced by using monoclonal κ, λ1, λ3, and λx proteins as standards. The monoclonal anti-$C_{\lambda 2}$ antibody does not discriminate between $C_{\lambda 2}$ and $C_{\lambda 3}$ (17). Since λx light chain contains the $C_{\lambda 2}$ region, the anti-$C_{\lambda 2}$ antibody detects λ2 and λ3 chains as well as λx chains. Anti-λx antibodies were prepared as follows. Rabbit anti-B6 (μ, λx) antiserum was extensively adsorbed on columns of B31 (μ, λ2) coupled to AH-Sepharose (Pharmacia). Specific anti-λx antibodies were purified by affinity chromatography on B8 (μ, λx) immunoglobulins.

RESULTS AND DISCUSSION

To increase production of λ-bearing immunoglobulins, BALB/c mice were treated with rabbit anti-λ2-LPS (17). Hybridomas were derived and one of them (B6) produced a λ-positive immunoglobulin that reacted with a monoclonal anti-$C_{\lambda 2}$ antibody (11). Blot hybridization analysis of RNA

Abbreviations: LPS, lipopolysaccharide; CDR, complementarity-determining (hypervariable) region; V, J, and C, variable, joining, and constant regions of immunoglobulin polypeptide chains (encoded by *V*, *J*, and *C* gene segments).

*This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03562)

showed that the B6 mRNA hybridized with a $C_{\lambda 2}$ probe but not with a V_λ probe. To characterize the light chain V region, a cDNA library was constructed from B6 poly(A)$^+$ mRNA and screened with the $C_{\lambda 2}$ probe. Insert size was analyzed in 33 clones that hybridized with the probe, and the clone (Y31) that contained the longest insert [0.9 kilobase (kb)] was sequenced as outlined in Fig. 1*a*. From the Y31 nucleotide sequence (Fig. 1*b*), four regions can be distinguished by analogy with the λ2 sequence. The *J* and *C* regions are identical to $J_{\lambda 2}$ and $C_{\lambda 2}$ germ-line sequences (8, 20). The *V–J* junction can be located between the codons GTG and TAT (amino acid residues 103 and 104), since it is known that the first four nucleotides of the $J_{\lambda 2}$ germ-line coding sequence are TTAT (20, 21). The leader sequence encodes a hydrophobic stretch of 19 amino acids and shows 61% nucleotide identity with the λ2 leader. The *V* segment (named $V_{\lambda x}$) shows 49% nucleotide identity with the $V_{\lambda x}$ gene segment and codes for 103 amino acids. Therefore the $V_{\lambda x}$ region is 6 amino acids longer than the other two V_λ regions (18, 19).

To determine possible phylogenetic relationships between the different V regions, we compared the $V_{\lambda x}$ amino acid sequence to known V sequences from the immunoglobulin gene superfamily. As shown in Fig. 2, the six canonical amino acids conserved in all V regions (28) are also found in $V_{\lambda x}$. According to Kabat *et al.* (29), nine other conserved residues are found in >95% of all V regions of immunoglobulin light chains. These amino acids are also conserved in $V_{\lambda x}$, except for an isoleucine that is replaced by methionine at position 54. A single nucleotide substitution could explain this replacement. Thus, the $V_{\lambda x}$ segment presents more homology with light chain V regions, including the human V_λ segment, than with any other known V region [i.e., immunoglobulin heavy chain (V_H) or T-cell receptor V regions]. However $V_{\lambda x}$ is not more homologous to $V_{\lambda 2}$ than to V_κ (33% v. 30% amino acid identity); furthermore, no preferential homology exists between this gene and any of the six human V_λ subgroups (29). Since members of V_κ or V_H gene clusters always show at least 45% identity (29, 30), the divergence between V_λ and $V_{\lambda x}$ is quite unprecedented. This suggests that the divergence of the $V_{\lambda x}$ gene segment from the common *V* light chain gene ancestor did not occur long after the phylogenetic separation between V_λ and V_κ genes.

Human and murine light chains usually exhibit a third hypervariable region (CDR3) composed of nine residues. In contrast, the $V_{\lambda x}$ carboxyl extremity contains four additional amino acids: -Glu-Gln-Phe-Val (EQFV in Fig. 2). If *D* (diversity) or *N* regions (38) were involved in λx expression, we would expect a certain degree of heterogeneity in the λx population. Several lines of evidence indicate that this is the case: light chains of all λx hybridomas tested gave

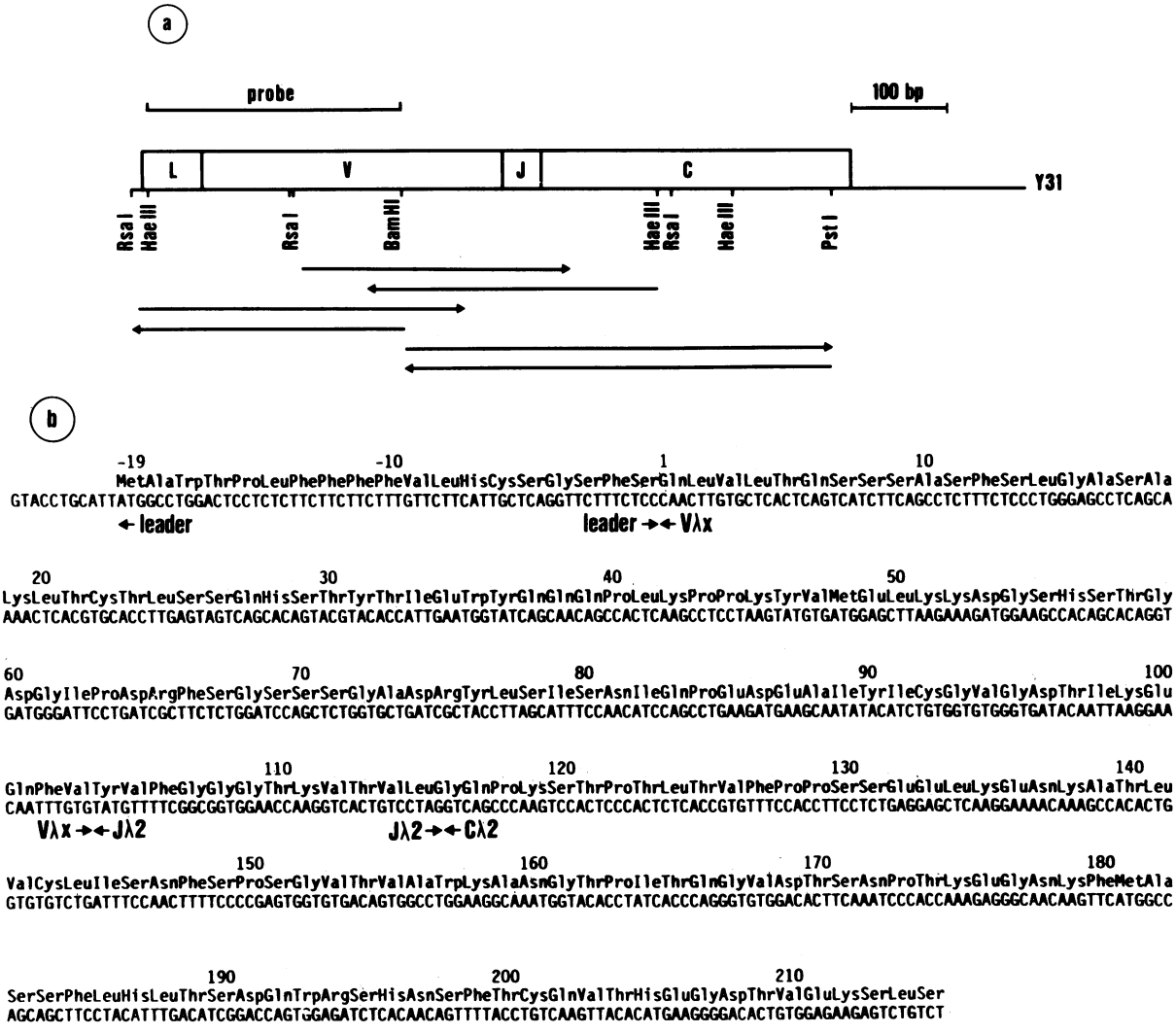

FIG. 1. Sequencing strategy and nucleotide sequence of the Y31 cDNA clone. (*a*) Restriction sites and strategy used for nucleotide sequence determination. Arrows indicate the direction and the length of determined sequence. (*b*) Nucleotide sequence of the Y31 cDNA clone and predicted amino acid sequence. L (leader), V, J, and C regions are delimited by arrows. Amino acid positions deduced by analogy to murine V_λ sequences (18, 19) are numbered from the start of the V region.

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                                        20                        40
                    *              •    |   •                     |•   •          *
Vλx           QLVLTQSSS/ASFSLGASAKLTCTLSSQ/////HSTYTIEWYQQQPLKPPKYVMEL
Vλ2           -A-V--E-A/LTT-P-GTVI---RS-TG//AVTT-N-AN/-V-EK-DHLFTGLIGG
VNEI          -SA---PA-/V-G-P-Q-ITIS--GTT///SDVG-YNFVS----N-G-A--LMIYE
Vk24          DI-I--DELSNPVTS-E-VSIS-RS-KSLLYKDGK--LN/-FL-R-GQS-QLLIYL
VH(MOPC104)   EVQ-Q--GP/QLVLP---VLMS-KA-GY////TFTD-YMK-VK-SHG-SLEWIGDI
VTα(TTII)     -QNVQ--PESLIVPE--RTS-N--F-DS/////A-Q-FW/--R-HSG-A--AL-SI
VTβ(86TI)     NTKI---PR/YLILGR-NKS-E-EQHLG///////HNAMY--K-SAE---ELMFLY
VTγ(8/10-2 II) --EQ-EL-V//TRETDENVQIS-IVYLP////YF-NTAIH--R-KTNQQFE-LIYV

                 60                80                     100
                 |           *    ** **          * *       | • •
Vλx           KKD/////GSHSTGDGIPDRFSGSSSGAD/RYLSISNIQPEDEAIYICGVGDTIKEQFV
Vλ2           TSN/////////RAP-V-V-----LI-DK/AA-T-TGA-T--D-M-F-ALWYSTH
VNEI          GNK/////////RPS-VSN-----K--KT/AS-T--GL-V----D-Y-CSYAGXST
Vk24          MST/////////RAS-VS------G--T-/FT-E--RVKA--VGV-Y-QQLVEYP
VH(MOPC104)   NPNNGGTSYNQKPKGKATLTVDK---T-///-MQLNSLTS--S-V-Y-AR
VTα(TTII)     FSN///////GEKEE-RFTIHLNKA-LH//FS-H-RDS--S-S-L-L-A-TLYGG
VTβ(86TI)     NLK//////QLIRNETV-S--IPECPDSSKLL-H--AVD---S-V-F-ASS
VTγ(8/10-2 II) ATN/YNQRPLGGKHKK-EASKDFK--TS///T-E-NYLKK----T-Y-A-WMR
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FIG. 2. Amino acid sequence of the $V_{\lambda x}$ region compared to various related V regions. Standard one-letter amino acid symbols are used. Gaps (/) were introduced to optimize alignment. Sequences of $V_{\lambda 2}$, human V_{λ} (VNEI), $V_{\kappa 24}$, heavy chain V region [VH (MOPC 104)], and V regions of T-cell receptor α [VTα (TT11)], β [VTβ (86T1)], and γ [VTγ (8/10-2 II)] polypeptides are drawn, respectively, from refs. 18 and 22–27. Dashes indicate identity with $V_{\lambda x}$. Dots indicate conserved amino acids in all V regions, according to Kronenberg *et al.* (28). Asterisks indicate conserved amino acids (>95%) in all V regions of light chains, according to Kabat *et al.* (29).

identical patterns upon isoelectric focusing (11), and in the C57BL/6 mouse, a λ light chain with the same CDR3 sequence was observed (12). Similar variability in length of CDR3 has been observed in rabbit V_{κ} regions (31). It has been assumed that this variability is based on length heterogeneity at the 3′ end of germ-line *V* gene segments (32). However, sequence determination of the germ-line $V_{\lambda x}$ gene would be necessary to assess possible participation of *D* segments or *N* elements.

Genomic organization of the $V_{\lambda x}$ gene was examined by blot analysis of restriction endonuclease-digested DNA (Fig. 3). When liver and SP2 DNAs digested by *Hin*dIII, *Bgl* II, and *Xba* I were hybridized with the $V_{\lambda x}$ probe, in all cases a single band (9, 6.2, and 2.6 kb, respectively) was detected. These fragments differ from those expected with V_{λ} and C_{λ} probes. The presence of a single fragment hybridizing with the $V_{\lambda x}$ probe was confirmed at low stringency (30 mM NaCl/3 mM sodium citrate, pH 7, 55°C; data not shown). This suggests that the $V_{\lambda x}$ gene is the only member of this gene family. An additional fragment was revealed with B6 DNA but not with

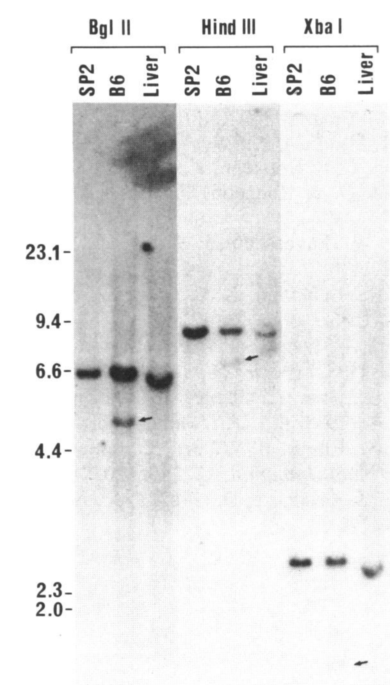

FIG. 3. Genomic analysis of the $V_{\lambda x}$ gene segment. DNA samples were digested by restriction endonucleases as indicated. Fragments hybridizing with $V_{\lambda x}$ probe were analyzed as described in *Materials and Methods*. The $V_{\lambda x}$ probe is a *Hae* III–*Bam*HI fragment as depicted in Fig. 1*a*. Fragment sizes were estimated by reference to λ-phage DNA digested by *Hin*dIII (marker sizes in kb at left). Arrows indicate the rearranged $V_{\lambda x}$ gene segment in the B6 hybridoma.

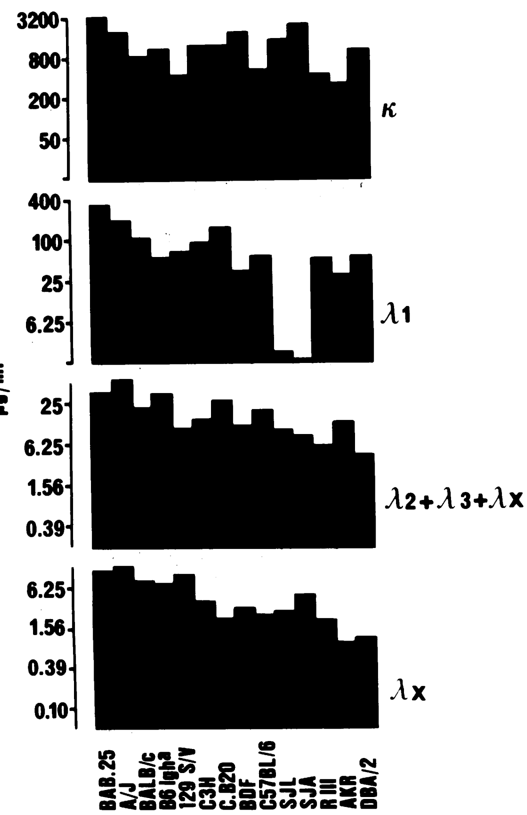

FIG. 4. Expression of light chain isotypes in normal sera of various mouse strains. Monospecific antibodies were used for determination of the κ, λ1, and λx concentrations. The monoclonal anti-$C_{\lambda 2}$ antibody used for λ2 + λ3 + λx detection, however, does not distinguish λ2, λ3, and λx light chains.

DNA from the SP2 fusion partner (Fig. 3), indicating that, as for other immunoglobulin chains, λx expression depends upon DNA rearrangements.

Since $V_{\lambda x}$ synthesis had thus far been studied only in BALB/c mice injected with rabbit anti-λ2-LPS, we tested its expression in normal sera of untreated inbred mice. Antibodies specific for λx were isolated from the serum of a rabbit immunized with polymerized B6 immunoglobulin. These antibodies detected λx in all normal sera tested. Fig. 4 indicates the amount of λx and other light chains found in sera of different mouse strains; λx expression levels vary in parallel with those of the $C_{\lambda 2}$ and $C_{\lambda 3}$ isotypes. These results are consistent with the data obtained with anti-λ2-LPS-induced hybridomas, where we found that out of 38 hybrids, 16 express λx, 14 express λ2, and 8 express λ3 light chains (ref. 11; P.S. and D. Juy, unpublished results). SJA and SJL mice have the $r^{lo}\lambda 1$ phenotype and express about 1% as much λ1 as do $r^{+}\lambda 1$ mouse strains (33, 34). However, the similar levels of λx expression observed in $r^{lo}\lambda 1$ and $r^{+}\lambda 1$ strains suggest that the $V_{\lambda x}$ gene segment may not be associated with $J_{\lambda 1}$–$C_{\lambda 1}$, and probably not with the $J_{\lambda 3}$–$C_{\lambda 3}$ gene pair either. We cannot exclude association with other, as yet unknown, *C* regions.

In wild mice, the λ genes have been found to be more numerous than in laboratory strains (35–37). However, this result was obtained by genomic DNA blot analysis using BALB/C λprobes. Our data raise the possibility that the λ system in wild and laboratory mice may be less different in complexity than previously supposed. The apparent disparity in gene number could have also resulted from differential evolution in wild and laboratory strains.

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1. Tonegawa, S. (1983) *Nature (London)* **302,** 575–581.
2. Eisen, H. N. & Reilly, E. B. (1985) *Annu. Rev. Immunol.* **3,** 337–365.
3. Elliot, B. W., Eisen, H. N. & Steiner, L. A. (1982) *Nature (London)* **299,** 559–561.
4. Reilly, E. B., Blomberg, B., Imanishi-Kari, T., Tonegawa, S. & Eisen, H. N. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2484–2488.
5. Weiss, S., Meyer, J. & Wabl, M. R. (1985) *Eur. J. Immunol.* **15,** 765–768.
6. Sakaguchi, N. & Melchers, F. (1986) *Nature (London)* **324,** 579–582.
7. Blomberg, B. & Tonegawa, S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 530–533.
8. Miller, J., Selsing, E. & Storb, U. (1982) *Nature (London)* **295,** 428–430.
9. Sakaguchi, N., Berger, C. N. & Melchers, F. (1986) *EMBO J.* **5,** 2139–2147.
10. Kudo, A., Sakaguchi, N. & Melchers, F. (1987) *EMBO J.* **6,** 103–107.
11. Sanchez, P. & Cazenave, P.-A. (1987) *J. Exp. Med.* **166,** 265–270.
12. Dildrop, R., Gause, A., Muller, W. & Rajewsky, K. (1987) *Eur. J. Immunol.* **17,** 731–734.
13. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25,** 263–269.
14. Bothwell, A. L. M., Paskind, M., Schwartz, R. C., Sonenstein, G. E., Gefter, M. L. & Baltimore, D. (1981) *Nature (London)* **290,** 65–67.
15. Messing, J. & Vieira, J. (1982) *Gene* **19,** 269–276.
16. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
17. Sanchez, P. & Cazenave, P.-A. (1986) *Eur. J. Immunol.* **16,** 895–900.
18. Tonegawa, S., Maxam, A. M., Tizard, R., Bernard, O. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1485–1489.
19. Bernard, O., Hozumi, N. & Tonegawa, S. (1978) *Cell* **15,** 1133–1144.
20. Selsing, F., Miller, J., Wilson, R. & Storb, U. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4681–4685.
21. Reilly, E. B., Reilly, R. M., Breyer, R. M., Sauer, R. T. & Eisen, H. N. (1984) *J. Immunol.* **133,** 471–475.
22. Graver, F. A. & Hilschmann, N. (1972) *Eur. J. Biochem.* **26,** 10–32.
23. Jouvin-Marche, E. & Rudikoff, S. (1986) *Immunogenetics* **24,** 191–201.
24. Kehry, M., Sibley, C., Fuhrman, J., Schilling, J. & Hood, L. E. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2932–2936.
25. Chien, Y., Becker, D. M., Lindsten, T., Okamura, M., Cohen, D. J. & Davie, M. M. (1984) *Nature (London)* **312,** 31–35.
26. Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. (1984) *Nature (London)* **308,** 153–158.
27. Iwamoto, A., Rupp, F., Onashi, P. S., Walker, C. L., Pircher, H., Joho, R., Hengartner, H. & Mak, T. W. (1986) *J. Exp. Med.* **163,** 1203–1212.
28. Kronenberg, M., Siu, G., Hood, L. E. & Shastri, N. (1986) *Annu. Rev. Immunol.* **4,** 529–591.
29. Kabat, A. E., Wu, T. T., Bilofoky, H., Raid-Miller, M. & Perry, H. (1983) *Sequences of Proteins of Immunological Interest* (Natl. Inst. Health, Bethesda, MD).
30. Patten, P., Yokota, T., Rothbard, J., Chien, Y., Arai, K. & Davis, M. M. (1984) *Nature (London)* **312,** 40–46.
31. Heidmann, O. & Rougeon, F. (1983) *Cell* **34,** 767–777.
32. Heidmann, O. & Rougeon, F. (1984) *Nature (London)* **311,** 74–76.
33. Geckeler, W., Faversham, J. & Cohn, M. (1978) *J. Exp. Med.* **148,** 1122–1136.
34. Epstein, R., Lehmann, K. & Cohn, M. (1983) *J. Exp. Med.* **157,** 1681–1686.
35. Scott, C. L., Mushinski, J. F., Huppi, K., Weigert, M. & Potter, M. (1982) *Nature (London)* **300,** 757–760.
36. Kindt, T. J., Gris, C., Guenet, J. L., Bonhomme, F. & Cazenave, P.-A. (1985) *Eur. J. Immunol.* **15,** 535–540.
37. Reidl, L. S., Elliott, B. W., Jr., & Steiner, L. A. (1986) *Curr. Top. Microbiol. Immunol.* **127,** 195–205.
38. Alt, F. W. & Baltimore, D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4118–4122.