activity), which inhibit Pol β and Pol γ. It is moderately sensitive to butylphenyl-guanosine triphosphate (10 μM, 72% activity; 100 μM, 14% activity). Other properties include a broad pH optimum for activity around pH 7.4 and only moderate sensitivity to salt (73% maximal activity at 0.15 M NaCl). The enzyme was relatively inactive in assays with activated salmon sperm DNA or primed homopolymers such as poly(dA):oligo(dT) or poly(dT):oligo(rA). The ratio of activity with these polymer templates relative to that obtained with the oligonucleotide template-primers is only 2 to 5% of the ratio obtained with yeast DNA Pol α. Pol ζ may be unusually sensitive to inhibition from nonproductive template binding, as was also suggested by its low activity on the M13 DNA template (Fig. 4). The existence of a nonessential DNA polymerase that is responsible for mutagenesis may have implications for the treatment of cancer and may provide opportunities for novel therapeutic strategies. Inhibition of a human homolog of *REV3*, if it exists, may be useful in patients who have been exposed to mutagenic agents or have an inherited predisposition to cancer.

REFERENCES AND NOTES

1. For a review, see E. C. Friedberg, G. C. Walker, W. Siede, *DNA Repair and Mutagenesis* (ASM Press, Washington, DC, 1995).
2. F. W. Larimer, J. R. Perry, A. A. Hardigree, *J. Bacteriol.* **171**, 230 (1989).
3. A. Morrison *et al.*, *ibid.*, p. 5659.
4. L. E. Torpey, P. E. M. Gibbs, J. R. Nelson, C. W. Lawrence, *Yeast* **10**, 1503 (1994).
5. S. Fields and O.-K. Song, *Nature* **340**, 245 (1989).
6. The two-hybrid pairwise test for protein interactions and the library screen for interacting proteins were carried out as described [S. M. Hollenberg, R. Sternglanz, P. F. Cheng, H. Weintraub, *Mol. Cell. Biol.* **15**, 3813 (1995)].
7. J. R. Nelson, C. W. Lawrence, D. C. Hinkle, data not shown.
8. Proofreading 3′ to 5′ exonuclease activity was assayed as described in Fig. 2, except that dNTPs were omitted and the 5′-labeled primer contained a single 3′-terminal nucleotide mismatch. No shortening of the primer was detected (<1 fmol) with up to 90 ng of Pol ζ or 3 ng of Pol α, nor was shortening observed with several other substrates (including two additional primers with mismatched ends, completely complementary primers, and single-stranded primers in the absence of template). In contrast, incubation of 200 fmol of one of these mismatched substrates with 0.1, 1, and 10 ng of Pol I Klenow fragment (which contains a 3′ to 5′ exonuclease) respectively shortened 2.5%, 25%, and 89% of the primers by 1 to 2 nt.
9. S. D. Rabkin, P. D. Moore, B. S. Strauss, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1541 (1983); G. L. Chan, P. W. Doetsch, W. A. Haseltine, *Biochemistry* **24**, 5723 (1985); K. L. Larson and B. S. Strauss, *ibid.* **26**, 2471 (1987).
10. M. Rajagopalan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10777 (1992).
11. J.-S. Taylor and C. L. O'Day, *Biochemistry* **29**, 1624 (1990).
12. C. L. O'Day, P. M. J. Burgers, J.-S. Taylor, *Nucleic Acids Res.* **20**, 5403 (1992).
13. J. R. Nelson, C. W. Lawrence, D. C. Hinkle, in preparation.
14. At each time and with both enzymes, 55 to 60% of the products were 16- to 18-nt oligomers, ~25% of the products were 19- to 32-nt oligomers, and 15 to 20% of the products were 33- to 200-nt oligomers.
15. DNA polymerase activity was determined as described in Fig. 2, except that the concentration of the competing nucleotide [deoxycytidine triphosphate (dCTP) for aphidicolin, deoxyguanosine triphosphate (dGTP) for butylphenyl-dGTP, deoxythymidine triphosphate (dTTP) for ddTTP, and dCTP for ddCTP) was lowered to 10 μM. Activity values are relative to activity without inhibitor.
16. K. Shimizu *et al.*, *J. Biol. Chem.* **268**, 27148 (1993).
17. P. Hovland, J. Flick, M. Johnston, R. A. Sclafani, *Gene* **83**, 57 (1989).
18. The plasmid pGST was created by moving a 1330–base pair (bp) Xba I–Sal I fragment containing the *GAL1* promoter and the gene encoding Gst from pRD56 [H.-O. Park, J. Chant, I. Herskowitz, *Nature* **365**, 269 (1993)] into YEplac195 [R. D. Geitz and A. Sugino, *Gene* **74**, 527 (1988)], which contains the 2μ replication origin and *URA3* for selection.
19. The plasmid pGST-REV3 was created by ligating a 4515-bp Eco RI–Sal I fragment containing *REV3* into pGST.
20. The plasmid pREV7 was constructed by ligating a 430-bp fragment containing the copper metallothionine gene promoter and a 747-bp fragment containing *REV7* into YEplac181, which contains the 2μ replication origin and *LEU2* for selection.
21. Rabbit polyclonal antibodies were generated against an SDS-PAGE–purified Rev3-TrpE fusion or Rev7p expressed in *E. coli*. Immunoblots were developed with goat antibody to horseradish peroxidase–conjugated rabbit secondary antibodies (Bio-Rad) and chemiluminescence (DuPont NEN).
22. The concentration of Gst-Rev3p was estimated by scanning a Coomassie blue–stained SDS–polyacrylamide gel and comparing the band intensities to the intensities of known amounts of bovine serum albumin (BSA).

23. We thank R. Bambara, M. Goodman, and R. Woodgate for comments; P. Ingles for initial work on the project; E. DiMuzio, M. Langer, and W. Sun for help with plasmid constructions; and M. Liskay and members of his lab for their help with the yeast two-hybrid screen. Supported by NIH grants GM21858 and GM29686.

4 December 1995; accepted 11 April 1996

A Quasi-Monoclonal Mouse

Marilia Cascalho, Audrey Ma, Steven Lee, Linda Masat, Matthias Wabl

As a model for studying the generation of antibody diversity, a gene-targeted mouse was produced that is hemizygous for a rearranged V(D)J segment at the immunoglobulin (Ig) heavy chain locus, the other allele being nonfunctional. The mouse also has no functional kappa light chain allele. The heavy chain, when paired with any lambda light chain, is specific for the hapten (4-hydroxy-3-nitrophenyl) acetyl (NP). The primary repertoire of this quasi-monoclonal mouse is monospecific, but somatic hypermutation and secondary rearrangements change the specificity of 20 percent of the antigen receptors on B cells. The serum concentrations of the Ig isotypes are similar to those in nontransgenic littermates, but less than half of the serum IgM binds to NP, and none of the other isotypes do. Thus, neither network interactions nor random activation of a small fraction of the B cell population can account for serum Ig concentrations.

The large diversity of antigen receptors on B cells hampers their study in intact mice. For several reasons discussed below, it is probably not possible to generate a truly monoclonal mouse—that is, a mouse in which every B cell expresses Ig molecules consisting of the same heavy (H) and light (L) chains—with a functional immune system. To study the generation of antibody diversity, we have therefore combined targeted gene replacement with mouse breeding to generate a mouse that is almost monoclonal. We call this animal the quasi-monoclonal (QM) mouse.

The extensively studied hapten NP induces an immune response that is restricted in the idiotype (*1*). Antibodies produced during primary responses are almost exclusively of the λ type. In particular, an Ig molecule consisting of a 17.2.25 heavy chain (*1*, *2*) combined with a λ light chain is specific for NP. In a conventional transgenic mouse line with a 17.2.25 transgene, μ_H17.2.25 combined with a λ1 light chain was shown to be specific for NP (*3*). In embryonic stem (ES) cells, we replaced the stretch of genomic DNA containing the J_H gene segments (*4*) with the V_HDJ_H 17.2.25 segment (Fig. 1A). Southern (DNA) blots were probed with μ and V_H 17.2.25 sequences to confirm that the introduced rearranged V(D)J segment was linked to C_μ and, hence, likely to be functional. Replacement mice obtained from the targeted ES lines were crossed to double "knockout" mice unable to express Ig heavy and κ chains because the J stretch had been deleted at both loci (*5*, *6*). The QM mice are those with the genotype V_HDJ_H 17.2.25/H^-, κ^-/κ^-, λ^+/λ^+ (Fig. 1B); they mimic the situation with a normal B lymphocyte, in which only one heavy and one light chain allele are expressed.

We used flow cytometry with the B220 and CD43 markers to analyze B cell development in spleen, bone marrow, and peripheral blood of QM mice. No gross abnormality was detected in these mice, with the exception

Department of Microbiology and Immunology, University of California, San Francisco, CA 94143–0670, USA.

that some had only one-third to one-half the number of precursor B and B lymphocytes present in their normal littermates. Because no such decrease was apparent in littermates with the V_HDJ_H 17.2.25 transgene and two intact κ chain alleles, and only a minor decrease was observed in those with one intact κ allele, we attribute the reduction in B cell numbers in the κ^-/κ^- mice to the fact that it is more difficult to generate a functional rearranged λ gene than a κ gene (*7*). Flow cytometry revealed that 8% of peripheral blood nucleated cells in QM mice are $B220^+$ B cells (Fig. 2A) and that 4.9% produce both IgM and IgD of the *a* allotype (the allotype of mouse strain 129, from which they are derived) (Fig. 2B). Thus, 85% of all the IgM^+ cells are also IgD^+, which is in the normal range. The μ-producing cells synthesize the λ chain (Fig. 2C) (*8*). The 1.7% of cells that are $\lambda^+\mu^-$ have presumably undergone the heavy chain class switch. Most B cells are V_H 17.2.25 idiotype–positive (4.3 + 1.5 = 5.8%)—that is, they express the V region exon introduced into the germ line. There are ~1.4% μ-positive, idiotype-negative cells (Fig. 2D).

There are two closely related V_λ gene segments, $V_\lambda1$ and $V_\lambda2$; $V_\lambda1$ can be joined to $J_\lambda1$ or can be joined to $J_\lambda3$, and $V_\lambda2$ can be joined to $J_\lambda2$ and, rarely, to $J_\lambda1$ or $J_\lambda3$ (*9*, *10*). In addition, there is a $V_\lambda X$, which associates with $J_\lambda2$ (*11*). Even if the same V_λ rearranges to the same J_λ, clones with independent rearrangements will differ in junctional diversity. This λ diversity is one of the reasons for naming our mouse "quasi" monoclonal. Although $V_\lambda1$ binds to NP when associated with $V_H17.2.25$, it was not known whether this is also true for $V_\lambda2$. We precipitated Ig from lipopolysaccharide-stimulated QM B cells with antigen and separated Ig chains on the basis of their different mobilities on an SDS-polyacrylamide gel (*10*). All three λ chains bound NP (*12*). The contribution of $V_\lambda X$ to the λ2 chain was not assessed (but it is not responsible for the idiotype-negative cells, as described below).

What fraction of the B cells actually bound the hapten NP? To label NP-binding cells in flow cytometry, we reacted peripheral blood cells first with NP coupled to bovine serum albumin (BSA) and then with fluorescein isothiocyanate (FITC)–coupled antibodies to human serum albumin that cross-react with

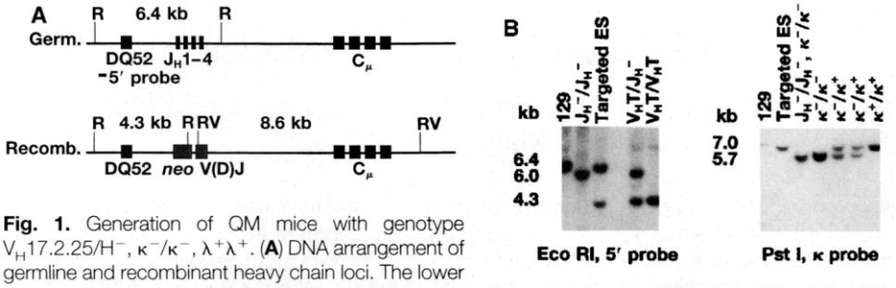

Fig. 1. Generation of QM mice with genotype $V_H17.2.25/H^-$, κ^-/κ^-, $\lambda^+\lambda^+$. (**A**) DNA arrangement of germline and recombinant heavy chain loci. The lower line also represents part of plasmid t-v.1, which was used to replace the J_H stretch in the ES cell line (*18*). R, Eco RI; RV, Eco RV. Fragment sizes generated by these enzymes are given. Gene (segments) encoding DQ52 J_H1–4, C_μ, neo^r, V(D)J, and the sequences covered by the 5′ probe are indicated. (**B**) Southern blot analysis of the heavy (left) and light (right) chain loci. The genotypes are given above the lanes: 129, ES line from strain 129; J_H^-/J_H^-, homozygous heavy chain knockout mouse; targeted ES, ES cell line in which one allele at the J_H locus was replaced by V_HT ($V_H17.2.25$); V_HT/J_H^-, mouse with one replaced and one nonfunctional heavy chain allele; V_HT/V_HT, mouse homozygous for the targeted replacement; J_H^-/J_H^-, κ^-/κ^-, double knockout mouse; κ^-/κ^-, κ^-/κ^+, and κ^+/κ^+, mice with genotype V_HT/H^- at the heavy chain locus and the given genotypes at the κ locus. Left panel, DNA digested with Eco RI; right panel, DNA digested with Pst I. The 5′ probe (left) was as indicated in (A), and the κ probe (right) was as in (*5*).

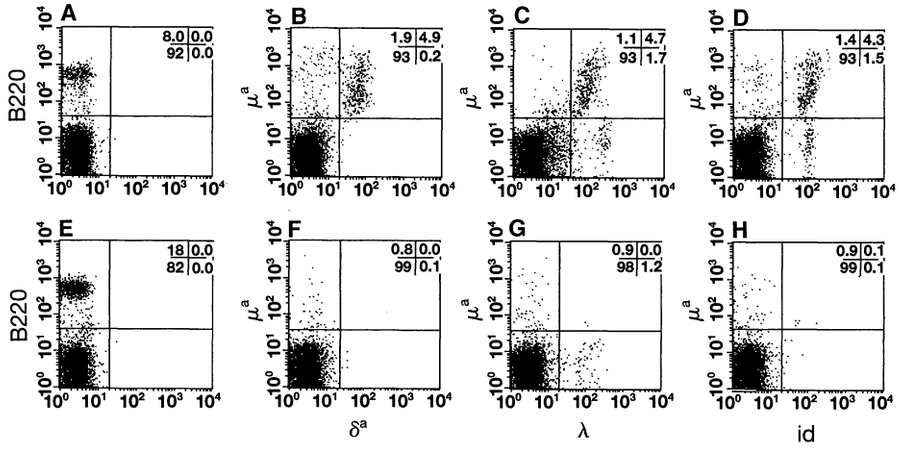

Fig. 2. Flow cytometric analysis of surface Ig on peripheral blood cells from a QM mouse (Ig^a haplotype) (**A** through **D**) and a C57BL/6 mouse (Ig^b haplotype) (**E** through **H**). Cells were stained with PE-coupled antibodies to B220 (anti-B220) [(A) and (E)], with PE-coupled anti-μ^a and FITC-coupled anti-δ^a [(B) and (F)], with PE-coupled anti-μ^a and FITC-coupled anti-λ (which reacts with λ1 and λ2) [(C) and (G)], and with PE-coupled anti-μ^a and FITC-coupled anti-$V_H17.2.25$ [anti-idiotype (id)] [(D) and (H)]. Ordinates and abscissas: log PE and FITC fluorescence intensities, respectively. Numbers in the upper right corner of each panel represent the percentage of cells in each quadrant.

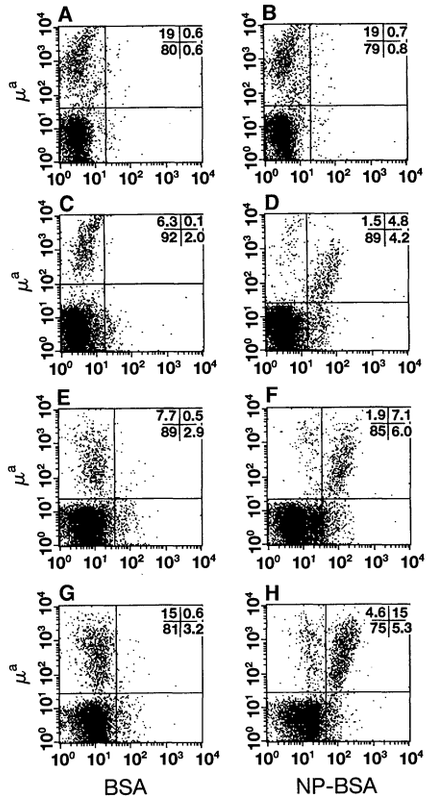

Fig. 3. Flow cytometric measurement of NP-binding B cells in a BALB/c mouse (Ig^a haplotype) (**A** and **B**) and QM mice 1 (**C** and **D**), 2 (**E** and **F**), and 3 (**G** and **H**). Peripheral blood cells were stained with BSA [(A), (C), (E), and (G)] or NP-BSA [(B), (D), (F), and (H)], and then with FITC-coupled antibodies to human serum albumin (which cross-react with BSA), to detect antigen-binding cells; they were also stained with a PE-coupled monoclonal antibody to μ^a to detect μ-bearing B cells. The PE-coupled antibodies were reacted after [(A) through (D)] or before [(E) through (H)] the FITC-coupled antibodies. Ordinates and abscissas: log PE and FITC fluorescence intensities, respectively. Numbers in the upper right corners of each panel represent the percentage of cells in each quadrant.

BSA. We used uncoupled BSA as a control. To label B lymphocytes, we incubated the cells with a phycoerythrin (PE)-coupled monoclonal antibody to μ^a (anti-μ^a). In a normal BALB/c mouse, few cells bound BSA (Fig. 3A) or NP-BSA (Fig. 3B). Virtually no B cells bound unconjugated BSA in QM mouse 1, which had 6.4% μ-staining cells (Fig. 3C) and at least 4.8% μ-producing, NP-binding cells (Fig. 3D); PE fluorescence intensity was decreased in the NP-binding cells of QM mouse 1, and as a result, the NP-positive cell population spilled over into the lower right quadrant. With cells from this mouse, as well as with cells of the BALB/c mouse, anti-μ^a was reacted after NP-BSA and anti-BSA, and we attribute the decrease in PE fluorescence intensity to steric hindrance of anti-μ binding by the NP-BSA and anti-BSA complex. When we reacted the cells first with anti-μ^a and then with the antigen, less hindrance was apparent, as seen in QM mice 2 (Fig. 3, E and F) and 3 (Fig. 3, G and H). Cells in the lower right quadrants of the panels in Fig. 3 should represent B cells that have switched their heavy chain; however, because of the spillover mentioned above and because of the high background values for μ-negative cells with uncoupled BSA, they are difficult to quantify. From 1.5 to 4.6% of the cells from the three QM mice expressed μ but did not bind NP. We confirmed these data by reacting the cells with antibodies specific for B220 and for NP. The ratio of NP-binding to non-binding cells was similar in all three mice. We estimate that ~80% of the μ-positive B cells bind NP in our QM mice.

To characterize the Ig receptors of those B cells that do not bind NP or the anti-idiotypic antibody, we sorted B220-positive, idiotype-negative cells, from which we isolated RNA and sequenced 10 independent V(D)J segments by reverse transcription and polymerase chain reaction analysis. In all 10 clones, V_H 17.2.25 had been replaced by another V_H in which a heptamer recombination signal sequence (RSS) was embedded near the 3′ end (*13*) (Fig. 4). That is, all 10 segments appeared to have undergone secondary rearrangement. Two of these clones (23 and 24) resemble the canonical V_H replacements—distinct from our experimental replacement of J_H with a preformed V_HDJ_H segment—that have been observed both in transformed cell lines (*14*) and in vivo (*15*). In this process, an exchange is mediated by the embedded heptamer RSS. The V_H in the preformed V_HDJ_H segment is replaced by a new V_H, often preserving a few base pairs of the original V_H.

Seven of the 10 clones appear to represent a new type of V_H replacement in which a new, rearranged V_HD segment has recombined with the D element of the original V_HDJ_H segment. In the remaining clone 7 (Fig. 4), the new V_HD element appears to have recombined with the J_H rather than with the D of 17.2.25. Why should V_HD replacement, which has not been detected in cell lines, be more common than V_H replacement in QM mice? At the rearranged heavy chain loci of normal mice, and of cell lines derived from them, all unused D segments are deleted during the rearrangement process; this is not the case when a preformed V_HDJ_H is experimentally inserted.

V_H81X is overrepresented in the embryonic repertoire and underrepresented in the adult. It has been suggested that V_H replacement accounts for at least part of this discrepancy. Although we and Chen *et al.* (*15*) have shown that secondary rearrangements can generate productive alleles, it is unclear to what extent V_H replacement contributes to antibody diversity in vivo. A normal mouse has a relatively diverse primary repertoire. There must be a far greater selection for almost any type of diversity in the QM mouse, which should make this animal a useful tool for revealing new mechanisms of diversity generation. However, it is not a good tool for quantifying the contribution of such mechanisms in normal animals. It has also been proposed that V_H replacement serves to edit out self-reactive antigen receptors (*16*). As the Ig in our QM mouse is not self-reactive, the observed V_H replacement does not contribute to, nor is it triggered by, receptor editing.

The increased selection for diversity in the QM mouse is also evident in the high frequency of mutations apparent in the sequences in Fig. 4. The six mutations in the nine intact J_H4 elements are separated from the secondary rearrangement breakpoints, even though it remains unclear whether the mutations and the rearrangements are independent of each other. The mutation frequency is thus 6 per 153 base pairs, or 4%, which would be an extremely high value

Clone	V_H	N	D	N	$D_{SP2.3}$	N	J_H4
V_HT	TACTGTGCTAGA				TACTATAGGTAC	CCT	TACTATGCTATGGACTA
5	TTCTGTGCAAGA	CGTCCT	TACTATGAT	CTA	TTCTATAGGTCC	CCT	TACTATGCTATGGACTA
37	TACTGTTCAAGA	ACG	NACTGGGAC	GGGG	ACTTTAATTAC	CCT	TACTATGCTATGGACTA
30	TACTGTGCTAGA	A	AACTGGGAC	GTGGACGG	AGGTAC	CCT	TACTATACTATGGACTA
8	TACTGTACAAGA	GAGG	GATTACGAC	TGTTA	TACTATAGGTAC	CCT	TACTATGTTATGGACTT
18	TACTGTGCAAGA	G	ACTATGATTACGAC	G	ATGGGTTC	CCT	TACTATGCTATGGANTT
29	TACTGTGCCAGAGA	GGG	ACTGGGGATCGCA		TACTATAGGTAC	CCT	TATTATGCTATGGACTA
35	TACTGTACAAGA	CAGGGG	TACGAC	GGGG	ACTATAGGTAC	CCT	TACTATGCTATGGACTA
7	TACTGTGCCAG	GCTA	TCTACTATGA	GGT			TGGACTA
23	TACTGTACAAGA			TATAGGG	GGTAC	CCT	TACTATGCTATGGACTA
24	TACTGTGNAAG			GGGCC	NGTAC	CCT	TACTATGCTCTGGACTA

Fig. 4. Secondary rearrangements in sorted B220-positive, idiotype-negative cells from the QM mouse. The sequence fragments shown start with the embedded heptamer near the 3′ end of V_H. V_HT, transgene 17.2.25. Mutations in $D_{SP2.3}$ and J_H4 are doubly underlined.

Table 1. Serum concentrations of Ig isotypes of six QM and five control mice. Isotype concentrations in sera of adult animals (9 to 20 weeks old) were calculated by comparing ELISA values to standard curves established for each isotype. NP-specific antibodies were assayed by coating plates with NP-BSA (1 μg/ml) in borate-buffered saline. NP-binding antibodies from hybridoma 17.2.25 (IgG1) were used as a positive control and as a standard for the IgG1 isotype.

Mouse	Serum concentrations (mg/ml) for isotype							
	IgM anti-NP	IgM	IgG1	IgG2a	IgG2b	IgG3	IgA	Σ Ig*
				C57BL/6 mice				
1	0	0.3	0.6	0.07	4.7	2.2	0.2	8.1
2	0	0.3	0.2	0.06	3.8	1.8	0.1	6.3
3	0	0.3	0.2	0.07	4.6	1.4	0.1	6.7
				BALB/c mice				
1	0	2.1	0.7	1.2	3.8	2.3	0.3	10.4
2	0	1.1	0.7	1.0	1.2	2.5	0.2	6.7
				QM mice				
1	0.2	0.5	0.6	2.5	1.5	2.7	1.1	8.9
2	0.1	0.6	0.4	3.2	3.8	2.6	1.4	12.0
3	0.1	0.3	1.0	2.8	3.3	2.5	0.8	10.7
4	0.2	0.5	1.0	4.8	3.4	1.6	0.8	12.2
5	0.4	0.9	1.6	8.0	7.1	1.2	1.1	19.9
6	0.5	1.9	0.9	11.1	9.9	6.3	2.7	32.8

*Total amount of serum Ig, with the exception of IgE.

even among antigen-binding cells after hyperimmunization, let alone in B cells from unimmunized animals. Even if the unstudied 80% idiotype-positive cells were devoid of mutations, the overall frequency would still be close to 1%.

With a standard enzyme-linked immunosorbent assay (ELISA), we determined the concentrations of various Ig isotypes in the sera of QM and control mice (Table 1). As expected, B cells in the QM mouse can and do switch from IgM to other isotypes. The data in Table 1 also provide clues to the origin of serum Ig in unimmunized animals, one of the oldest unsolved problems in immunology. If network interactions among surface receptors (*17*) were necessary to produce serum Ig, its concentration should be low in QM mice. However, QM mice tended to have higher concentrations of the various isotypes, especially IgG2a and IgA, than did control animals housed under the same conditions. QM mice 5 and 6 were mildly and highly hypergammaglobulinemic, respectively. Less than half the IgM and none of the other isotypes tested bound NP, confirming the flow cytometric data showing that monospecificity evolves into diversity in these mice. Cells with mutant receptors appear to be preferentially expanded and switched. We can also exclude a simple stochastic model in which serum Ig would be a consequence of random activation of a small fraction of B cells. Such a model would predict that the fraction of serum Ig that binds NP would reflect the fraction of B lymphocytes that bind NP. Although the B cell content of the entire mouse was not surveyed, most B lymphocytes in peripheral blood and spleen bind NP, and thus the stochastic model would predict that most of the serum Ig would bind NP. In summary, we can rule out a purely network model and a purely random stochastic model for the origin of serum Ig. Although serum Ig represents a record of previous antigenic exposure, some type of homeostatic control must also play a role.

REFERENCES AND NOTES

1. T. Imanishi and O. Mäkelä, *J. Exp. Med.* **140**, 1498 (1974); K. Karjalainen, *Eur. J. Immunol.* **10**, 132 (1980); M. E. White-Scharf and T. Imanishi-Kari, *ibid.* **11**, 897 (1981); *ibid.* **12**, 935 (1982).
2. D. Y. Loh, A. L. M. Bothwell, M. E. White-Scharf, T. Imanishi-Kari, D. Baltimore, *Cell* **33**, 85 (1983).
3. R. Grosschedl, D. Weaver, D. Baltimore, F. Constantini, *ibid.* **38**, 647 (1984).
4. S. Taki, M. Meiering, K. Rajewsky, *Science* **262**, 1268 (1993).
5. J. Chen *et al.*, *EMBO J.* **12**, 821 (1993).
6. J. Chen *et al.*, *Int. Immunol.* **5**, 647 (1993).
7. D. A. Ramsden and G. E. Wu, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10721 (1991).
8. Approximately 5.6% of the cells express λ1 and 2.4% express λ2, yielding a λ1/λ2 ratio of 70/30, as determined by PE-coupled anti-μ^a and FITC-coupled anti-λ1 and anti-λ2. In a C57BL/6 control mouse, the λ1/λ2 ratio was 76/24. Because the binding site of the monoclonal anti-λ1 is not known, λ3 is detected together with either λ1 or λ2.
9. E. Appella, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 590 (1971); E. P. Schulenberg, E. S. Simms, R. G. Lynch, R. A. Bradshaw, H. N. Eisen, *ibid.*, p. 2623; T. Azuma, L. A. Steiner, H. N. Eisen, *ibid.* **78**, 569 (1981); B. Blomberg, A. Traunecker, H. N. Eisen, S. Tonegawa, *ibid.*, p. 3765; J. Miller, A. Bothwell, U. Storb, *ibid.*, p. 3829; E. Selsing, J. Miller, R. Wilson, U. Storb, *ibid.* **79**, 4681 (1982); B. W. Elliot, H. N. Eisen, L. A. Steiner, *Nature* **299**, 559 (1982); S. Carson and G. E. Wu, *Immunogenetics* **29**, 173 (1989); E. B. Reilly, B. Blomberg, T. Imanishi-Kari, S. Tonegawa, H. N. Eisen, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2484 (1984).
10. S. Weiss, J. Meyer, M. R. Wabl, *Eur. J. Immunol.* **15**, 765 (1985).
11. R. Dildrop, A. Gause, W. Muller, K. Rajewsky, *ibid.* **17**, 731 (1987); P. Sanchez and P. A. Cazenave, *J. Exp. Med.* **166**, 265 (1987).
12. Material precipitated by sequential incubation of cells with NP conjugated to BSA and antibodies to BSA contained the same polypeptide chains—μ, λ1, and λ2—as material precipitated with an antiserum to μ and λ. No such material was precipitated with NP-BSA from cells of a C57BL/6 mouse nor with BSA lacking the hapten NP from cells of QM or C57BL/6 mice. These results confirm our flow cytometric data showing that most B cell receptors in the QM mouse bind antigen.
13. Only the 3′ end with the embedded heptamer of each V_H is shown in Fig. 4. Except for a few base pair differences, the new V_H segments correspond with known V_H sequences in the Kabat *et al.* [E. A. Kabat, T. T. Wu, H. M. Perry, K. S. Gottesman, C. Foeller, *Sequences of Proteins of Immunological Interest* (NIH, Bethesda, MD, ed. 5, 1991)] and GenBank databases. The lack of identity is likely attributable to hypermutation and the fact that the new V_H segments are derived from mouse strain 129, whereas those in the databases are derived from other strains, principally BALB/c. These details are currently being resolved; in the present context, it is important only that they are not similar to V_H17.2.25.
14. R. Kleinfield *et al.*, *Nature* **322**, 843 (1986); M. Reth, P. Gehrmann, E. Petrac, P. Wiese, *ibid.*, p. 840; G. B. Beck-Engeser, H.-M. Jäck, M. R. Wabl, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1060 (1987); R. W. Kleinfield and M. G. Weigert, *J. Immunol.* **142**, 4475 (1989); L. R. Covey, P. Ferrier, F. W. Alt, *Int. Immunol.* **2**, 579 (1990); S. Usuda *et al.*, *EMBO J.* **11**, 611 (1992); T. Shirasawa *et al.*, *J. Exp. Med.* **176**, 1209 (1992); T. Komori, Y. Minami, N. Sakato, H. Sugiyama, *Eur. J. Immunol.* **23**, 517 (1993); E. J. Steenbergen *et al.*, *Blood* **82**, 581 (1993).
15. C. Chen, Z. Nagy, E. L. Prak, M. Weigert, *Immunity* **3**, 747 (1995).
16. S. L. Tiegs, D. M. Russell, D. Nemazee, *J. Exp. Med.* **177**, 1009 (1993); D. Gay, T. Saunders, S. Camper, M. Weigert, *ibid.*, p. 999.
17. N. K. Jerne, *Annal. D. Immunol.* **125C**, 373 (1974); A. Coutinho, *Scand. J. Immunol.* **42**, 3 (1995); F. Varela *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 5917 (1991).
18. The 16.3-kb plasmid t-v.1 contains a rearranged μ_H chain gene, without the membrane exons, and the selectable markers *neo*^{r} and *tk*, which are both in an orientation opposite that of the inserted anti-NP μ gene. The 17.2.25 V region contains V_H10 joined to $D_{SP2.3}$ and J_H4; it is flanked by DNA sequences homologous to those 5′ and 3′ of the stretch containing the J_H segments in the normal heavy chain locus. The 3′ sequence contains the major intron and C_μ. The shorter, 5′ sequence contains DQ52, the most proximal D segment, and flanking sequences. The cloning strategy was as follows: The 5′ homologous region, a 1.6-kb Hind III–Bam HI DNA fragment containing DQ52, was cloned into the same sites of Pmc1Neo (Stratagene). Subsequently, the Xho I site 5′ of the DQ52 segment was destroyed by nucleotide incorporation with the Klenow fragment and blunt-end ligation. The Xho I–Sal I herpes simplex virus–thymidine kinase (*HSVtk*) gene was then cloned into the remaining Xho I site. The Hind III site 5′ of DQ52 was also eliminated to allow cloning of the heavy chain gene [the Sal I–Xho I fragment from plasmid pμ (*3*)] into the Hind III site 5′ of the *HSVtk* gene.

19. We thank L. Reichardt and G. Martin for help with producing the QM mouse; R. Grosschedl for the plasmid pμ; T. Imanishi-Kari for providing antibodies to V_H17.2.25 and NP-BSA; D. Huszar and N. Lonberg of GenPharm for providing the heavy chain and κ double knockout mice; R. Murray and P. Vieira for advice on generating replacement mice; and C. Steinberg for editorial assistance. Supported by NIH grants 1R01 GM37699 and P60 AR20684, the Engalitcheff Award of the Arthritis Foundation, the Lucille P. Markey Charitable Trust, the Junta Nacional de Investigação Científica e Tecnológica–Praxis XXI-BD 3763/94 (M.C.), and the American-Italian Foundation for Cancer Research (L.M.).

26 December 1995; accepted 18 April 1996

Identification of MAP Kinase Domains by Redirecting Stress Signals into Growth Factor Responses

Anne Brunet and Jacques Pouysségur

Mitogen-activated protein kinase (MAPK) cascades, termed MAPK modules, channel extracellular signals into specific cellular responses. Chimeric molecules were constructed between p38 and p44 MAPKs, which transduce stress and growth factor signals, respectively. A discrete region of 40 residues located in the amino-terminal p38MAPK lobe directed the specificity of response to extracellular signals, whereas the carboxyl-terminal half of the molecule specified substrate recognition. One p38-p44MAPK chimera, expressed in vivo, redirected stress signals into early mitogenic responses, demonstrating the functional independence of these domains.

MAP kinases form a large family of serine-threonine protein kinases activated by separate cascades conserved through evolution (*1*). In mammalian cells, three distinct MAPK cascades have been identified: p42-p44 MAPKs (*2*), p38 MAPK (*3*, *4*), and p46-p54 JNKs (*5*). Activation of p42-p44 MAPKs constitutes a crucial step in the pathway mediating cell proliferation in re-

Centre de Biochimie–CNRS, UMR134, Parc Valrose, Faculté des Sciences, 06108 Nice Cedex 2, France.