Targeted Insertion of a Variable Region Gene into the Immunoglobulin Heavy Chain Locus

Shinsuke Taki,* Myriam Meiering, Klaus Rajewsky†

A mutant mouse strain has been generated in which a rearranged immunoglobulin heavy (H) chain variable (V) region gene is placed into the heavy chain locus in its natural position, replacing the J_H elements. In homozygous mutant mice, essentially all B cells in the spleen express the transgenic V_H region in their antibodies. The proper location of the transgene relative to the constant region genes allows it to participate in isotype switching and undergo somatic hypermutation. Immunoglobulin transgenic mice generated in this fashion by gene targeting should prove useful for the exploration of immunoregulatory mechanisms.

Immunoglobulin (Ig) transgenic mice expressing a single antibody specificity in the great majority of their B cells have been successfully used in studies of various aspects of immunoregulation such as the control of allelic exclusion of antibody V region genes (*1*) and of immunological tolerance (*2*). However, in such animals transgene expression is affected to various extents by the endogenous Ig loci. Furthermore, the switch from the expression of one antibody class to that of another (*3*) is usually either impossible or severely restricted (*4*). The different antibody classes are involved in mediating distinct effector functions in the antibody response (*5*). In addition, in the pathogenesis of antibody-mediated autoimmune diseases, class switching of autoantibodies associated with somatic hypermutation of V region genes may often play a critical role (*6*).

The gene targeting technique (*7*), used in the mouse almost exclusively to inactivate functional genes in the germline, also provides a way to place any given functional gene or other DNA sequence into a particular position in the genome. We designed a targeting vector in order to introduce a rearranged V_H region gene [V_HT15; derived from an antibody to phosphorylcholine (anti-PC) (*8*)] into a chromosomal position where rearranged V_H genes locate, 5′ to the heavy chain enhancer (Fig. 1, A and B) (*9*). A successful targeting event would yield an IgH locus carrying the V_HT15 gene in the position of J_H (preventing further J_H rearrangements) and a *neo*^{r} gene 5′ of the V_H gene in an inverted orientation (Fig. 1B). A mutant embryonic stem (ES) cell clone (T15i#5-2) was identified by polymerase chain reaction (PCR) (*10*) and analyzed by Southern (DNA) blotting to confirm that the mutation had been correctly introduced (Fig. 1C). We obtained chimeric mice by injecting T15i#5-2 cells into C57BL/6-derived blastocysts, and the mice transmitted the mutant chromosome into their offspring (Fig. 1C). We designate the mutation carried by these mice *T15i*.

The *T15i* chromosome is derived from an ES cell, the IgH loci of which are of the IgH^a allotype. Because the mutant chromosome was crossed into strain C57BL/6 ($IgH^{b/b}$), B cells expressing the *T15i* locus should carry antibodies of allotype *a* on the cell surface. Furthermore, the same cells should be stainable by an antibody to the idiotype specific for the V_HT15 product (V_HT15Id) (*11*). Figure 2 depicts the flow cytometric results obtained from the staining of peripheral blood lymphocytes (PBLs) from homozygous ($IgH^{T15i,a/T15i,a}$) and heterozygous ($IgH^{T15i,a/b}$) mutants as well as wild-type ($IgH^{b/b}$) littermate mice with antibodies to the Ig μ chain *a* and *b* allotypes on one hand and V_HT15Id and *a* allotype on the other. As expected, essentially all μ^a-positive cells carry the V_HT15Id in both homozygous and heterozygous mutants. Such cells are absent in the controls and dominant in the homozygous mutant. Notably, we observed the presence of a large population (~60% of the B cells) of μ^b-positive, V_HT15Id-negative cells in the blood of heterozygous mutants. On the basis of the allelic exclusion principle (*1*, *12*), one would have expected that in the course of B cell development in these animals, the (productive) V_H region gene rearrangement on the *T15i* chromosome would inhibit the other chromosome from undergoing V_H gene rearrangement. Allelic exclusion appears to operate in the heterozygous mutant B cells, because they express either μ^a (together with V_HT15Id) or μ^b (lacking V_HT15Id). This suggests that in the μ^b-expressing cells the *T15i* locus is inactivated through secondary gene rearrangements. For reasons that remain to be explored, such rearrangements are rare or absent in homozygous mutant B cells as revealed by Southern blotting of genomic

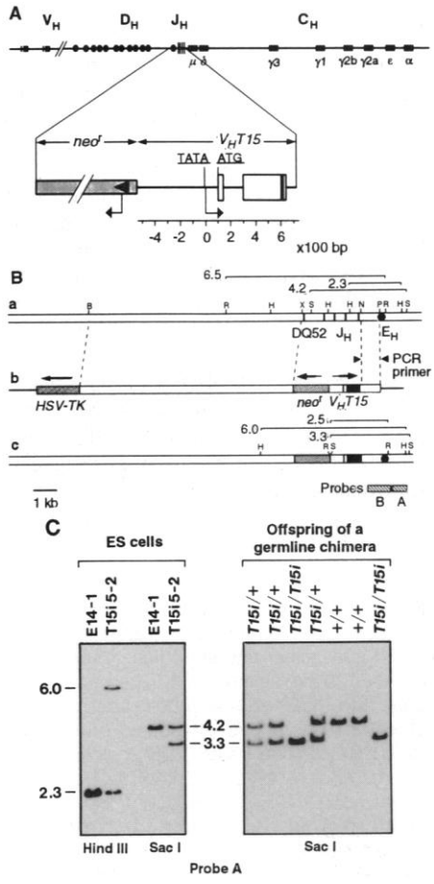


Fig. 1. Targeted insertion of the V_HT15 gene into the germline IgH locus. (**A**) Genomic structure of the wild-type IgH locus and the targeted insertion. D_H, J_H, and C_H regions are shown as circles, vertical lines, and filled boxes, respectively. Arrows indicate transcriptional initiation sites and orientation. (**B**) Strategy for homologous recombination. (a) Partial restriction map of the wild-type J_H locus. Exons for DQ52 and J_H's are shown as narrow filled boxes and the H chain enhancer as a filled circle. B, Bam HI; R, Eco RI; H, Hind III; X, Xho I (the only unique site); S, Sac I; N, Nae I; P, Pvu II. (b) Targeting construct (*9*). PCR primers are shown as triangles. Horizontal lines represent plasmid sequence. Arrows indicate transcriptional orientation of the *neo*^{r} and the V_HT15 gene. (c) Predicted structure of the targeted IgH (*T15i*) locus. Diagnostic restriction fragments and location of probes used for Southern blot analysis are shown. (**C**) Southern blot analysis of a PCR-positive transfectant and offspring of germline chimeras. Hind III– and Sac I–digested genomic DNA of a clone (T15i#5-2) of the ES line was hybridized to probe A. The parental ES cell line (E14-1) was included as a control. With both enzymes, clone T15i#5-2 shows a hybridizing fragment in addition to that derived from the wild-type IgH locus. Sac I–digested tail DNA from offspring of heterozygous mutant animals was hybridized to the same probe as for ES cell DNA. Homozygous mutant mice ($IgH^{T15i,a/T15i,a}$) show only a 3.3-kb hybridizing fragment, whereas heterozygous mutants ($IgH^{T15i,a/b}$) show both a 4.2- and 3.3-kb fragment. Sizes are indicated in kilobases.

Institute for Genetics, University of Cologne, D-50931, Cologne, Germany.

*Present address: Basic Research Laboratories, Ajinomoto Company, Inc., Kawasaki 210, Japan.
†To whom correpondence should be addressed.

DNA with a J_H probe (*13*). This is in accord with an important conclusion from the flow cytometry data in Fig. 2; specifically, that B cells expressing V_H genes other than V_HT15 are generated in homozygous mutant mice at very low frequency, if at all.

The spleen of the homozygous mutant mice harbors normal numbers of T and B lymphocytes (*14*), and essentially all B cells express the V_HT15Id. However, the spleen of the homozygous T15i mutant mice lacks the $IgM^{low}IgD^{high}$ B cell population (Fig. 3A), similar to an earlier observation in a conventional IgM- and IgD-expressing Ig transgenic mouse (*15*). This phenomenon could be due to either intrinsic properties of the particular transgenes in question or to cellular selection or activation, or both, on the basis of the specificities of the transgenic antibody.

The V_HT15 gene used for targeted insertion was taken from the myeloma cell line S107 which produces an anti-PC (8). This antibody is produced almost exclusively by $CD5^+$B (B1) cells in normal mice (*16*). However, B1 cells, as characterized by the CD5 surface marker, were not only virtually absent in the spleen of the homozygous mutant mice but also rare in the peritoneal cavity (Fig. 3B). This contrasts with conventional Ig transgenic mice in which the H chain transgene encodes another B1 cell–associated antibody specificity, where large numbers of $CD5^+$ B cells are recruited into both peritoneal cavity and spleen (*17*).

Does class switching occur on the *T15i* chromosome? The serum of homozygous T15i mice contained antibodies of all Ig isotypes examined, with somewhat higher IgM concentrations and lower IgG and IgA concentrations as compared with the control (Fig. 4). Essentially all serum IgM appears to express V_HT15Id (Fig. 5), in contrast with the situation in conventional Ig transgenic mouse strains where much of serum IgM is derived from endogenous IgH loci (*1*, *18*). However, the titers of V_HT15Id-bearing IgG1, IgG3, and IgA antibodies appear to be lower than those of total IgG1, IgG3, and IgA, respectively. This effect may be due to the occurrence of somatic hypermutation in the corresponding cells, as a single amino acid exchange may abolish reactivity to antibody to V_HT15Id (anti-V_HT15Id) (*19*).

The above results demonstrated that the targeted *IgH* allele can undergo class switching, although the frequency of class switching on the targeted chromosome might be lower than that on a wild-type chromosome. Such an effect could be due to the presence of the neo^r gene 5′ of the V_H promoter in the *T15i* locus. Moreover, V_HT15-expressing B cells might be less likely to be stimulated to class switching by environmental antigens than normal B cells

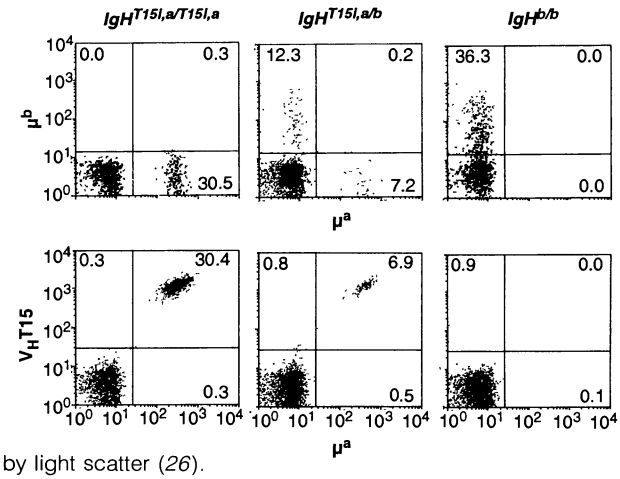


Fig. 2. Allotype and idiotype of surface IgM on B cells in the peripheral blood of homozygous and heterozygous mutant mice and control mice. PBLs were isolated from mice of the F2 generation and stained with fluorescein isothiocyanate (FITC)–conjugated monoclonal antibody (mAb) to μ^a and phycoerythrin (PE)–conjugated mAb to μ^b (*25*). Biotinylated mAb to V_HT15 (mAb TC54) was used to identify V_HT15-expressing B cells (*11*). Numbers in quadrants refer to the percentage of cells in the lymphocyte gate as defined by light scatter (*26*).

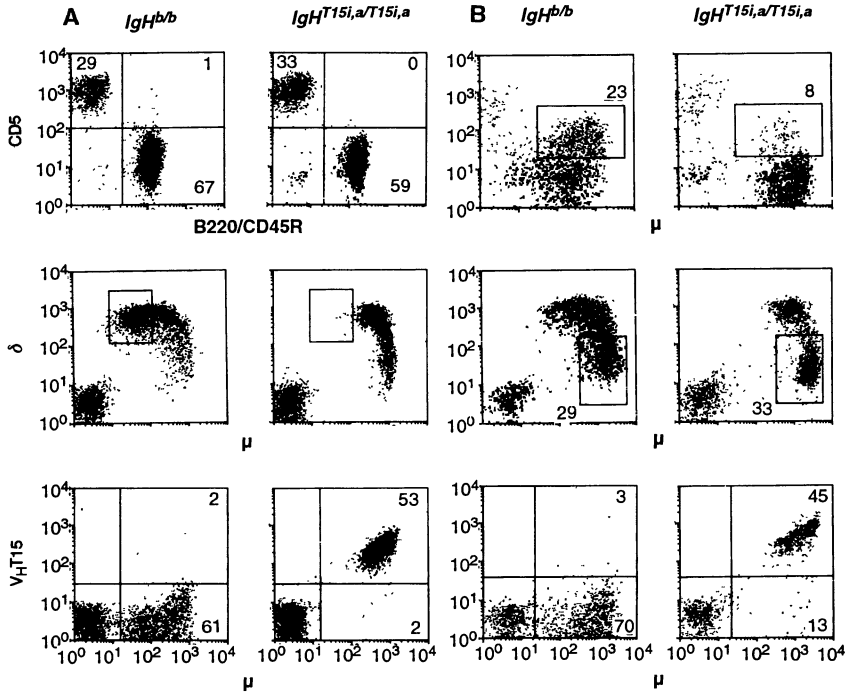
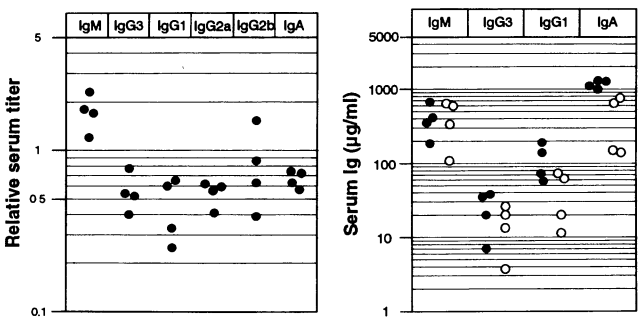


Fig. 3. Surface phenotype of splenic (**A**) and peritoneal (**B**) B cells from wild-type ($IgH^{b/b}$) and homozygous T15i mutant 8-week-old mice ($IgH^{T15i,a/T15i,a}$). Cells were stained with FITC-conjugated antibodies as indicated (*27*). In (A), boxes in dot plots of the middle row denote $IgM^{dull}IgD^{bright}$ B cells. In (B), $CD5^{dull}$ and $IgM^{bright}IgD^{dull}$ cells are boxed, and the percentages of cells in a given box are shown for each plot. Numbers refer to cells in the lymphocyte gate.

Fig. 4 (left). Relative concentrations of Ig isotypes in the blood of 7-week-old homozygous T15i mutant mice, determined by enzyme-linked immunosorbent assay (ELISA) (*28*). Titers are expressed relative to those of the wild-type littermates. Each filled circle represents a single animal. **Fig. 5 (right).** Antibodies bearing V_HT15Id in the blood of homozygous T15i mutant mice. V_HT15Id-bearing (○) and total antibody (●) titers were determined for IgM, IgG3, and IgG1. Concentrations of V_HT15Id-bearing and total antibodies were calculated with V_HT15Id-positive monoclonal standards for each isotype (*28*).



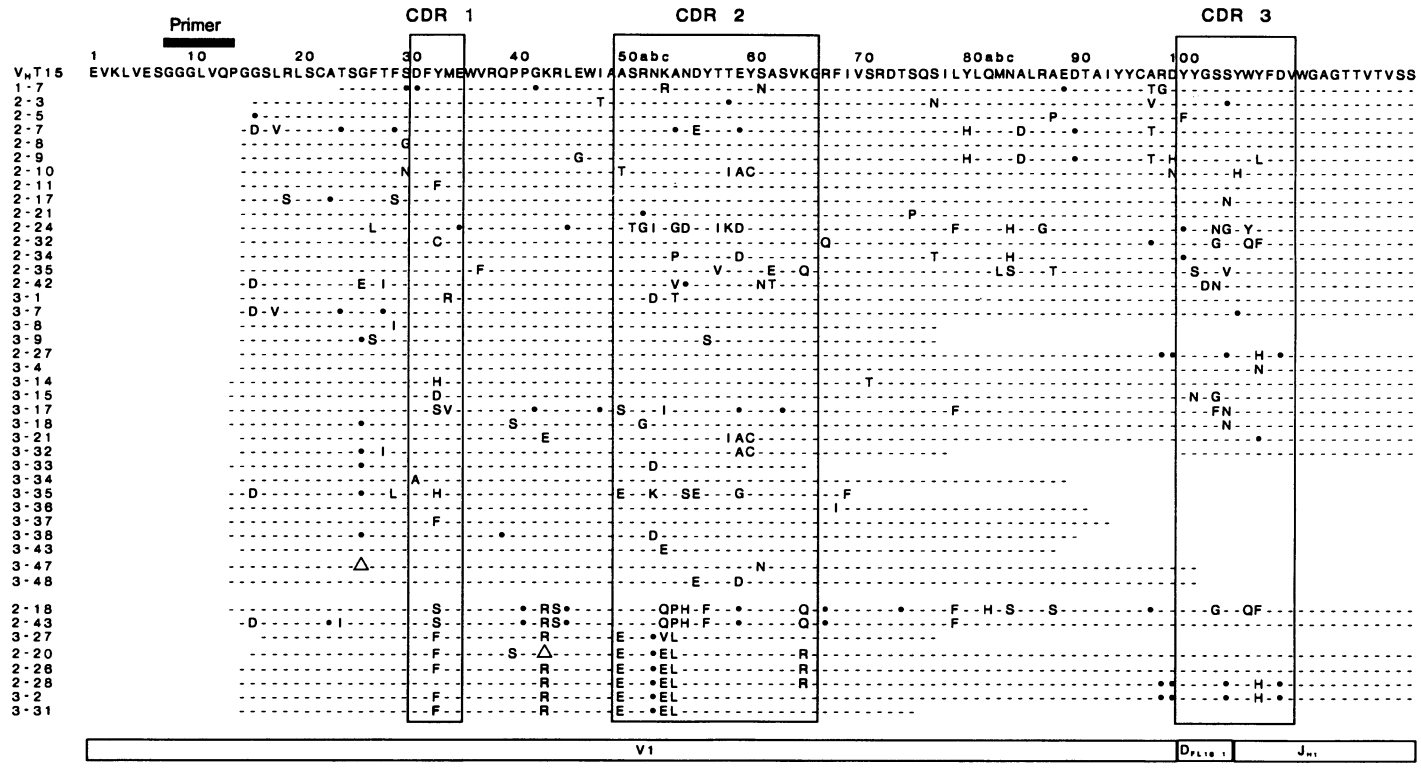


Fig. 6. Somatic hypermutation of the transgenic V_HT15 gene obtained from IgM$^-$IgD$^-$ B cells of PC-KLH–immunized homozygous T15i mutant mice (*29*). Deduced amino acid sequences are compared with that of the V_HT15 gene. Codons are numbered according to Kabat *et al.* (*30*). Dashes indicate identity. Amino acid substitutions are shown, and codons containing silent mutations and base deletions are represented by dots and triangles, respectively (*31*).

because of their restricted antibody repertoire. Because of these possibilities, we assessed class switching on the *T15i* allele in vitro, in the absence of any influence of external antigens that are known to be the dominant force driving class switching in vivo (*20*). Mouse B cells cultured with lipopolysaccharide (LPS) alone or with LPS and interleukin-4 (IL-4) show class switching mainly to IgG3 or IgG1, respectively (*21*). This occurred in splenic B cells from homozygous T15i mice (most of which express IgM) [Fig. 3 and (*13*)] as efficiently as in cells from wild-type mice (Table 1). Thus, the lower concentrations of IgG and IgA in the serum of the homozygous mutants (Fig. 4) may result from the impact of a restricted antibody repertoire on cellular selection.

Table 1. Percent of splenic B cells producing μ or δ chains from T15i homozygous mice. Splenic B cells from homozygous mutant ($IgH^{T15i,a/T15i,a}$) and control mice were cultured in vitro (*24*) with either LPS or LPS and IL-4. On day 6 of culture, B cell blasts were purified on a Ficoll density gradient, fixed, and analyzed for cytoplasmic Ig by staining with isotype-specific antibodies. Values represent the percent of positive B cell blasts.

Mice	LPS		LPS and IL-4	
	μ$^+$	γ3$^+$	μ$^+$	γ1$^+$
	$IgH^{T15i,a/T15i,a}$			
1	44.4	10.6	39.5	28.9
2	46.5	15.9	39.2	20.8
3	48.7	10.0	52.5	31.3
	$IgH^{b/b}$			
1	47.1	16.0	31.6	31.9
2	38.1	18.8	54.5	21.1
3	40.5	11.2	32.1	30.3

For the analysis of somatic hypermutation (*22*) of the inserted V_HT15 transgene, we isolated IgM$^-$IgD$^-$ B cells from the spleens of homozygous mutant mice after they had been immunized with an appropriate T cell–dependent antigen [PC-coupled keyhole limpet hemocyanin (PC-KLH)]. From the mRNA of these cells, V_HT15 transcripts associated with sequences from IgG constant regions were specifically amplified by PCR, cloned, and sequenced (*23*). Most of the 44 nucleotide sequences (depicted in Fig. 6) bore a multitude of somatic point mutations with an average of 5.7 mutations per sequence and a preferential accumulation of replacement mutations in complementarity-determining regions (CDRs)—similar to what analysis in normal IgM$^-$IgD$^-$ B cells revealed (*23*). As in normal mice, IgM-expressing B cells in homozygous mutants express largely unmutated V_HT15 genes (*13*). We conclude from these results that the V_HT15 transgene in the T15i mutant strain undergoes somatic hypermutation to the same extent as do rearranged V_H genes in normal mice, with T cell–dependent antigenic stimulation.

The present data establish a method of generating "second generation" Ig transgenic mice, in which the transgene behaves like a normal rearranged Ig gene in terms of B cell–specific expression, class switching, and somatic hypermutation. The method is not only applicable to any rearranged V_H gene, but can be easily extended to light chain genes as well. Transgenic mice of this kind, generated by gene targeting, should provide useful mouse models for the in vivo analysis of antigen-specific B cell activation and tolerance, including models of allergic and autoimmune diseases.

REFERENCES AND NOTES

1. S. Rusconi and G. Köhler, *Nature* **314**, 330 (1985); D. Weaver, F. Costantini, T. Imanishi-Kari, D. Baltimore, *Cell* **42**, 117 (1985); M. C. Nussenzweig *et al.*, *Science* **236**, 816 (1987); J. Manz, K. Denis, O. Witte, R. Brinster, U. Storb, *J. Exp. Med.* **168**, 1363 (1988).
2. C. C. Goodnow, *Annu. Rev. Immunol. 10*, 489 (1992); D. Nemazee *et al.*, *Immunol. Rev.* **122**, 117 (1991).
3. A. Shimizu and T. Honjo, *Cell* **36**, 801 (1984).
4. J. Durdik *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2346 (1989); R. M. Gerstein *et al.*, *Cell* **63**, 537 (1990)

5. D. R. Burton and J. M. Woof, *Adv. Immunol.* **51**, 1 (1992).
6. R. Papoian, R. Pillarisetty, N. Talal, *Immunology* **32**, 75 (1977); A. Kohno *et al.*, *Immunogenetics* **18**, 513 (1983); M. Schlomchik *et al.*, *Nature* **328**, 805 (1987); B. Diamond *et al.*, *Annu. Rev. Immunol.* **10**, 731 (1992); S. Taki *et al.*, *Eur. J. Immunol.* **22**, 987 (1992).
7. M. R. Capecchi, *Science* **244**, 1288 (1989).
8. P. N. Anderson and M. Potter, *Nature* **222**, 994 (1968); M. Cohn, G. Notani, S. A. Rice, *Immunochemistry* **6**, 111 (1969); R. Perlmutter *et al.*, *Adv. Immunol.* **35**, 1 (1984). The rearranged V_H and V_κ genes of the antibody S107 are identical to those of the antibody TEPC15. We call the rearranged V_H gene of S107 *V_HT15*.
9. A 9-kb Bam HI–Xho I DNA fragment upstream of DQ52 was subcloned as a 2-kb Bam HI–Xho I fragment isolated from phage clone ChSpμ27 [P. Early *et al.*, *Cell* **19**, 981 (1980)] and a 7-kb Bam HI fragment from cosmid clone COS-J17 [C. Wood and S. Tonegawa, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3030 (1983)]. The *V_HT15* gene spanning from a Bam HI site 0.6 kb upstream of the ATG codon to a Bgl II site 60 bp downstream of *J_H1* was isolated from pLLg3R [J. W. Guise, P. L. Lim, D. Yuan, P. W. Tucker, *J. Immunol.* **140**, 3988 (1988)] and ligated to an 0.8-kb Nae I–Pvu II fragment upstream of the heavy chain intron enhancer element. The targeting vector was assembled with the *HSV-tk* gene from pIC19R/MC1-tk [S. L. Mansour, K. R. Thomas, M. R. Capecchi, *Nature* **336**, 348 (1988)] and the *neo*-cassette as modified by Jung *et al.* (FRT-*neo*^{r}-FRT cassette) (*24*). The final construct was linearized with Pvu I at a unique site in the pUC19 vector sequence and used for transfection.
10. The E14-1 cells, a subclone of the ES cell line E14 [M. Hooper, K. Hardy, A. Handyside, S. Hunter, M. Monk, *Nature* **326**, 292 (1987)] were transfected with the linearized vector and double-selected with G418 and Gancyclovir (GANC) as described [R. Kuhn, K. Rajewsky, W. Muller, *Science* **254**, 707 (1991)]. We found only one clone (#5-2) that was positive by PCR screening, with the primers shown in Fig. 1B, out of more than 400 double-resistant clones.
11. C. Desaymard, A. M. Giusti, M. D. Scharff, *Mol. Immunol.* **21**, 961 (1984).
12. S. Tonegawa, *Nature* **302**, 79 (1983); F. Alt *et al.*, *EMBO J.* **3**, 1209 (1987); D. Kitamura and K. Rajewsky, *Nature* **356**, 154 (1992).
13. S. Taki, M. Meiering, K. Rajewsky, unpublished results.
14. We noticed that the relative number of peripheral B cells of F2 mice varied substantially from animal to animal, probably because of genetic background variations. As a result of the mating procedure, the genetic background of mice of the F2 generation is a mixture between 129/Ola and C57BL/6.
15. R. Brink *et al.*, *J. Exp. Med.* **176**, 991 (1992).
16. H. Masmoudi, T. Mota-Santos, F. Huetz, A. Coutinho, P.-A. Cazenave, *Int. Immunol.* **2**, 515 (1990); S. Taki *et al.*, *Ann. N.Y. Acad. Sci.* **651**, 328 (1992).
17. K. Hayakawa, C. E. Carmack, S. A. Shinton, R. R. Hardy, *Ann. N.Y. Acad. Sci.* **651**, 346 (1992). As shown in Fig. 3B, the percentages of $IgM^{bright}IgD^{dull}$ B cells in the peritoneal cavity of homozygous mutant mice are not different significantly from those of wild-type mice. This observation might indicate that the $IgM^{bright}IgD^{dull}$ B cells seen in the peritoneal cavity and the spleen of homozygous mutant mice are B1 cells lacking the CD5 surface marker (B1b cells) [L. A. Herzenberg, A. B. Kantor, L. A. Herzenberg, *ibid.*, p. 1].
18. R. Grosschedl, D. Weaver, D. Baltimore, F. Costantini, *Cell* **38**, 647 (1984).
19. N. C. Chien, R. Pollock, C. Desaymard, M. D. Scharff, *J. Exp. Med.* **167**, 954 (1988).
20. H. Hooijkaas, R. Benner, J. R. Pleasants, B. S. Wostmann, *Eur. J. Immunol.* **14**, 1127 (1984).
21. C. Esser and A. Radbruch, *Annu. Rev. Immunol.* **8**, 717 (1990).
22. C. Kocks and K. Rajewsky, *ibid.* **7**, 537 (1989); C. Berek and C. Milstein, *Immunol. Rev.* **96**, 23 (1987).
23. B. Schittek and K. Rajewsky, *J. Exp. Med.* **176**, 427 (1992).
24. S. Jung, K. Rajewsky, A. Radbruch, *Science* **259**, 984 (1993).
25. Lymphocytes were isolated from peripheral blood by Ficoll density gradient and stained with mAbs before analysis on FACScan (Becton Dickinson). The following mAbs were used: FITC-conjugated mAb to μ^a [RS3.1; R. Schüppel, J. Wilke, E. Weiler, *Eur. J. Immunol.* **17**, 739 (1987)], PE-conjugated mAb to μ^b [MB86, S. Nishikawa *et al.*, *Immunogenetics* **23**, 137 (1986)], and biotin-conjugated mAb to V_HT15 [TC54; (*11*)]. The biotin-conjugated antibodies were developed by PE-streptavidin.
26. I. Förster, P. Vieira, K. Rajewsky, *Int. Immunol.* **1**, 321 (1989).
27. The following antibody conjugates were used: FITC-conjugated mAb to B220/CD45R [RA3-6B2; R. L. Coffman, *Immunol. Rev.* **69**, 5 (1982)], biotin-conjugated mAb to CD5 (Becton Dickinson), FITC-conjugated goat antibody to δ (Nordic Immunological Laboratories, Capistrano Beach, CA), FITC-conjugated mAb to μ [R. Grützmann, thesis, University of Cologne (1981)], and biotin-conjugated mAb to V_HT15. Biotin conjugates were developed with streptavidin-PE or Cy-Chrome (PharMingen).
28. Isotype-specific ELISA was done as described [D. Kitamura *et al.*, *Cell* **69**, 823 (1992)]. Standard antibodies expressing V_HT15Id were BH8 for IgM [J. F. Kearney *et al.*, *Eur. J. Immunol.* **11**, 877 (1981)], S107γ3 for IgG3, and S107γ1 for IgG1. The isotype variants, S107γ3 and S107γ1, were purified from the supernatants of hybridoma cell lines carrying expression vectors for the *V_HT15* gene linked to mouse Cγ3 and Cγ1 genes for mouse, respectively (*32*). The parent cell line used for transfection carried an expression vector for the κ light chain of antibody TEPC15/S107 with V_κ22 and C_κ isolated from plasmid pLLg3R (*9*).
29. Eight-week-old mice homozygous for the *T15i* mutation were immunized with PC-KLH as described (*16*). On day 15 after immunization, spleen cells were prepared and IgM^-IgD^- $B220^+$ cells were purified as described (*23*). We amplified *V_HT15* sequences from complementary DNA with a Cγ primer, 5′-CAGAATTCGGATCCCAGGGGCCAGTGGATAGAC-3′ (*23*), and a *V_HT15* primer, 5′-GGAGGAAGCTTGGTACAGCCTGG-3′ (restriction sites are underlined) [A. Feeney, *J. Exp. Med.* **172**, 1377 (1990)]. Amplified DNAs were cloned and nucleotide sequences determined.
30. E. A. Kabat *et al.*, *Sequences of Proteins of Immunological Interest* (Public Health Service, Department of Health and Human Services, Bethesda, MD, 1991).
31. Nucleotide sequence data are availale from European Molecular Biology Laboratory under accession number DS15953.
32. M. Meiering and S. Taki, unpublished results.

33. We thank D. Kitamura, R. Kühn, J. Roes, S. Jung, W. Müller, E. Sonoda, and B. Schittek for discussion and advice; Genetic Institute for a LIF-producing cell line; P. W. Tucker, L. Hood, and C. Wood for DNA; J. F. Kearney, M. D. Scharff, and A. Coutinho for antibodies; P. W. Tucker for pLLg3R; C. Uthoff-Hachenberg, R. Zoebelein, and C. Göttlinger for technical assistance; and U. Ringeisen and W. Müller for work on graphs. The animal work was done in accord with official regulations. Supported by the Deutsche Forschungsgemeinschaft through SFB 243, the Federal Ministry for Research and Technology, the Land Nordrheinwestfalen, the Human Frontier Science Programme, and the Fazit Foundation.

26 July 1993; accepted 1 October 1993

Generation of a Mouse Strain That Produces Immunoglobulin κ Chains with Human Constant Regions

Yong-Rui Zou, Hua Gu, Klaus Rajewsky

Humanized antibodies are highly efficient as immunotherapeutic reagents and have many advantages over rodent antibodies. A mouse strain was generated by gene targeting to replace the mouse κ light chain constant (C) region gene with the human C_κ gene. Mice homozygous for the replacement mutation (C_κR) produced normal concentrations of serum antibodies, most of which carry chimeric κ light chains, and mounted normal immune responses to hapten-protein conjugates. This technology provides a feasible option for the generation of high-affinity humanized antibodies by means of the powerful somatic hypermutation-selection mechanism.

Various technologies have been developed to overcome problems related to the production of human monoclonal antibodies (mAbs) (*1*), one of which is the generation of chimeric antibodies in which the rodent C regions of heavy (H) and light (L) chains [with or without the framework of the variable (V) region] are replaced by the equivalent domains or sequences of human immunoglobulin (Ig) (*2*). Another strategy attempts to mimic the immune response in vitro through the expression of human Ig V region genes (isolated from human B cell populations or immunodeficient mice engrafted with human lymphoid tissue) in bacteriophages, followed by selection for rare, high-affinity antibodies through antigen binding (*3*). A major drawback to these and similar approaches is the cumbersome work required to generate each specific mAb of appropriate biological function.

An ideal solution to these problems would be the generation of a mouse strain that synthesizes human instead of mouse antibodies. This has been approached by

Institute for Genetics, University of Cologne, 50931 Cologne, Germany.