# Contribution of Receptor Editing to the Antibody Repertoire

**Rafael Casellas,[1] Tien-An Yang Shih,[1] Markus Kleinewietfeld,[3] Jasna Rakonjac,[1]* David Nemazee,[4] Klaus Rajewsky,[3] Michel C. Nussenzweig[1,2]†**

**Receptor editing, clonal deletion, and anergy are the mechanisms by which B cells maintain tolerance to self antigens. To determine the extent to which receptor editing shapes the normal antibody repertoire, we generated an immunoglobulin κ polymorphism that facilitates the detection of editing of immunoglobulin light chains in vivo. We found that B cells are targeted for editing during a 2-hour delay in development at the pre-BII cell stage, and that about 25% of all antibody molecules are produced by gene replacement. These results suggest that receptor editing represents a major force in shaping the antibody repertoire.**

The clonal selection theory anticipated that a random collection of immunoglobulins (Igs) would include self-reactive specificities that require silencing (*1*). The mechanisms by which such autoreactive B cells are tolerized were subsequently uncovered using transgenic mice carrying antibody genes coding for self-reactive Igs (*2–6*). In these mice, autoreactive B cells confronted with self antigens were eliminated, anergized, or altered by continued gene recombination, a process known as receptor editing (*2–6*). For example, nearly all B cells from mice carrying recombined antibodies to double-stranded (ds) DNA or to major histocompatibility complex have their autoreactive specificities replaced by receptor editing (*7, 8*). There is also indirect evidence from Southern blotting and DNA sequence analysis that editing occurs in nontransgenic B cells (*9–11*). However, the role of editing in shaping the antibody repertoire under physiological conditions is unknown.

To measure the extent to which editing occurs in developing B cells in vivo, we generated an allelic polymorphism of the mouse κ constant region (mCκ) by replacing it with the human counterpart (hCκ) [Fig. 1A, $Igκ^{m/h}$ (*12*)]. $Igκ^{m/h}$ mice showed 43% hCκ, 44% mCκ, and 5% double-expressing B cells by flow cytometry (Fig. 1A). When individual double producers were isolated and their Igκ genes amplified and sequenced, we found that only 1.5% of B cells in $Igκ^{m/h}$ mice expressed two Igκ chains (*12*). We conclude that Igκ allelic exclusion is a highly efficient process, and that there is no gross selective bias against hCκ expression in $Igκ^{m/h}$ mice.

To determine whether the hCκ marker can be used to detect receptor editing in vivo, we used mouse models in which the extent of editing is well defined (*7, 13*). The hCκ allele was introduced into mice carrying antibodies specific for either dsDNA ($IgH^{3H9/+}Igκ^{Vκ4/h}$) or single-stranded (ss) DNA ($IgH^{3H9/+}Igκ^{Vκ8/h}$), or an antibody with no apparent self-reactivity ($IgH^{B1-8/+}Igκ^{αHEL/h}$) (*7, 12, 13*). In agreement with previous work, we found little editing in B cells carrying the innocuous antibody or the antibody to ssDNA, because almost all B lymphocytes in $IgH^{B1-8/+}Igκ^{αHEL/h}$ and $IgH^{3H9/+}Igκ^{Vκ8/h}$ mice expressed the pre-recombined mCκ allele [Fig. 1B, columns 1 and 2 (*7*)]. In contrast, nearly all of the B cells in mice that carry the antibody to dsDNA underwent receptor editing [Fig. 1B, column 3 (*7*)]: 49% of the B cells in $IgH^{3H9/+}Igκ^{Vκ4/h}$ mice expressed hCκ, and the original Vκ4 allele was rarely found by mRNA analysis in the $mCκ^+$ cells [Fig. 1B, column 3, and Table 1 (*7, 14*)]; the remaining 10 to 12% of the B cells in $IgH^{3H9/+}Igκ^{Vκ4/h}$ mice expressed Igλ. We conclude that expression of hCκ correlates with previous measurements of receptor editing. Furthermore, the extent of editing of $IgH^{3H9}$ antibodies depends on the light chain because B cells expressing $IgH^{3H9}Igκ^{Vκ8}$ antibodies are not edited, whereas B cells expressing $IgH^{3H9}Igκ^{Vκ4}$ antibodies are nearly entirely edited.

To investigate whether receptor editing in $Igκ^{m/h}$ mice is induced by changes in the antibody combining site, we generated mice carrying two $IgH^{B1-8}$ variants detected during the immune response to 4-hydroxy-3-nitrophenylacetyl (NP) (*15*). Replacement of $Trp^{33}$ by Leu in complementarity-determining region CDR1 of $IgH^{B1-8}$ increases the affinity for NP by a factor of 10 ($IgH^{B1-8high}$), whereas the four amino acid changes found in hybridoma 3C52 decrease NP binding by a factor of 4 ($IgH^{B1-8low}$) (*15*). Each of these naturally occurring mutations, in combination with the αHEL light chain, converts an apparently innocuous antibody that is not edited into a receptor that induces a great deal of editing (Fig. 1B, columns 4 and 5). About 38% of B cells in $IgH^{B1-8high/+}Igκ^{αHEL/h}$ mice and 64% of B cells in $IgH^{B1-8low/+}Igκ^{αHEL/h}$ mice expressed hCκ on their cell surface. Increased Igλ usage in the mouse B cell repertoire has often been used as a marker for receptor editing (*6, 10, 16*). However, increased Igλ expression was only seen in $IgH^{B1-8high}$ mice (Fig. 1B). The low level of surface Igλ expression in $IgH^{B1-8low}$ B cells is not due to the inability of this heavy chain to pair with λ light chains, because $IgH^{B1-8low}Igλ$ is a naturally occurring antibody combination (*15*). Thus, surface Igλ expression does not always correlate with receptor editing.

In addition to self-reactivity, it has been suggested that abnormally high levels of antibody expression, and possibly poor pairing of heavy and light chains, may also induce receptor editing (*17, 18*). Because the structure of all three $IgH^{B1-8}$ targeted genes is identical, different IgH expression levels are not likely to be responsible for the difference in editing seen between B1–8 and its two variants. In addition, the B1–8 mutations do not interfere with the assembly of the heavy chains with the αHEL light chain (*12*). Thus, it appears that the $B1–8^{high}$ and $B1–8^{low}$ heavy chains produce self-reactive antibodies when combined with VκαHEL. We conclude that $Igκ^{m/h}$ mice can be used to detect gene replacement triggered by changes in the antigen combining site.

To determine the extent of receptor editing for a single light chain combined with any random heavy chain, we introduced the hCκ allele into mice expressing the pre-recombined VκαHEL light chain. In these mice, Ig heavy chain gene recombination is not constrained. Therefore, VκαHEL light chains, expressed at the pre-BII cell stage, are paired with the full spectrum of mouse heavy chains, an unknown

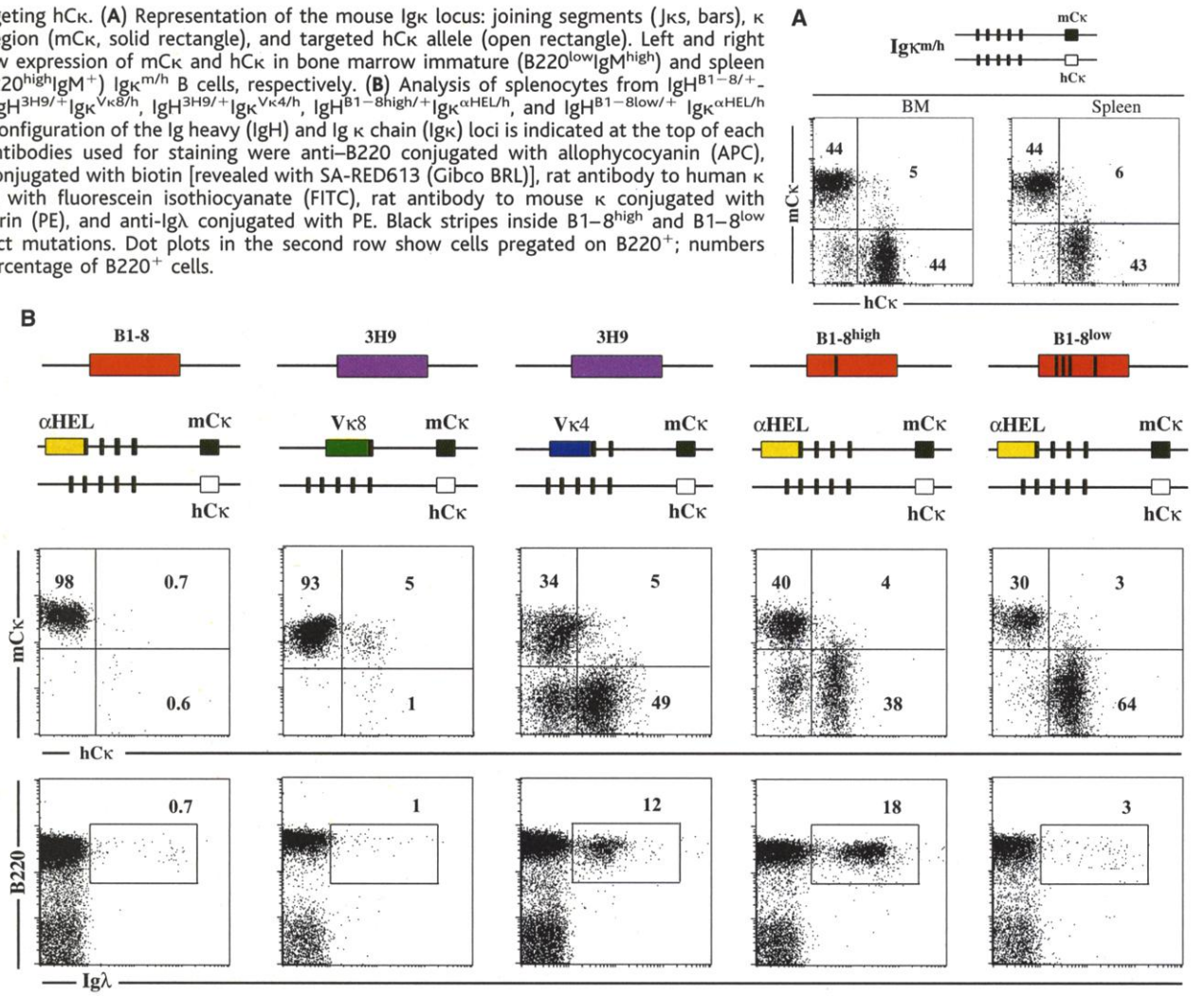
**Table 1.** Receptor editing on targeting alleles. $mCκ^+$ cells from $IgH^{3H9/+}Igκ^{Vκ4/h}$, $Igκ^{αHEL/h}$, and $Igκ^{Vκ4/h}$ mice were sorted and their Vκ-Jκ (mCκ) genes isolated and sequenced.

| Mouse | Number of samples | mCκ/Vκ trans-gene$^+$ | mCκ/Vκ trans-gene$^-$ |
|---|---|---|---|
| $IgH^{3H9/+}Igκ^{Vκ4/h}$ | 42 | 1 | 41 |
| $Igκ^{αHEL/h}$ | 37 | 34 | 3 |
| $Igκ^{Vκ4/h}$ | 36 | 34 | 2 |

[1]Laboratory of Molecular Immunology, [2]Howard Hughes Medical Institute, Rockefeller University, New York, NY 10021, USA. [3]Institute for Genetics, University of Cologne, Weyertal 121, 50931 Cologne, Germany. [4]Department of Immunology, Scripps Research Institute, La Jolla, CA 92037, USA.

*Present address: Massey University, Palmerston North, New Zealand.

†To whom correspondence should be addressed. E-mail: nussen@rockvax.rockefeller.edu




number of which would be expected to induce receptor editing. B cells that undergo receptor editing and replace the original mouse VκαHEL allele were detected by a combination of flow cytometry and mRNA analysis. We found that VκαHEL light chains are frequently replaced during B cell development. Analysis of bone marrow Igκ$^{αHEL/h}$ B cells shows that 14% (±2) of B lymphocytes in these mice express hCκ either alone or in combination with mCκ, and 3% (±1) express λ chains (Fig. 2A). The extent of editing on the mCκ locus was determined by cloning and sequencing mIgκ mRNAs from sorted mCκ$^+$ B cells. We found that 8.5% of the Igκ mRNAs from purified mCκ$^+$ lymphocytes were products of receptor editing (6% of the B cells, Table 1). Therefore, about 22% of newly formed B cells in Igκ$^{αHEL/h}$ mice replace the targeted light chain.

Two mechanisms have been proposed to explain receptor editing in transgenic models (*2, 6–8*). Editing might be specifically induced by self-reactive or nonpairing receptors. Alternatively, editing could reflect random premature Vκ-Jκ recombination in pro-B cells (*19*) followed by deletion of remaining autoreactive B cells (*2, 6–8*). To determine whether editing is random or specifically induced, we compared the kinetics of development of B cells that do or do not undergo light chain gene replacement in vivo. Igκ$^{αHEL/h}$ mice and controls were injected with a single dose of the thymidine analog 5-bromo-2′-deoxyuridine (BrdU), which is incorporated into the DNA of large pre-BII cells that are in the S phase of the cell cycle (*20*). Large pre-BII cells are the immediate precursors of small pre-BII cells, noncycling cells that actively rearrange their Ig light chain genes and become immature B cells (IgM$^+$) (*21*) (Fig. 2B). The time elapsed between BrdU injection and the first appearance of labeled immature B cells corresponds to the minimum time spent in the small pre-BII compartment. In agreement with previous experiments, BrdU-labeled immature B cells first appeared after 4.5 hours in Igκ$^{m/h}$ control mice irrespective of the Igκ allele expressed on the cell surface (hCκ or mCκ) [Fig. 2B (*22–24*)]. Therefore, it normally takes a minimum of 4.5 hours for cells to go from a germ line Igκ locus in large pre-BII cells to cell surface expression of a functional Igκ in immature B cells. In contrast, immature B cells expressing the pre-recombined VκαHEL light chain (mCκ$^+$) emerged 2.6 hours after BrdU injection in Igκ$^{αHEL/h}$ mice (Fig. 2B). The time difference between the pre-recombined VκαHEL B cells and their wild-type counterparts is consistent with the completion of $G_2$ and mitosis phases of the cell cycle by large pre-BII cells and suggests that B cells with innocuous receptors spend little or no time in the small pre-BII stage (*24*).

In contrast to unedited VκαHEL-expressing B cells (mCκ$^+$), all of the edited B cells from the same mice (hCκ$^+$) were developmentally delayed, and the first edited cells appeared in the immature compartment after 4.5 hours (Fig. 2B, right panel; similar results were obtained with Igκ$^{3\text{-}83/h}$ and Igκ$^{Vκ8/h}$ mice). This difference in kinetics indicates that B cells undergoing editing are specifically delayed in the small pre-BII cell compartment for at least 2 hours. Thus, the rate of transit through the small pre-BII cell compartment correlates with receptor editing. We con-

**Fig. 1.** Targeting hCκ. (**A**) Representation of the mouse Igκ locus: joining segments (Jκs, bars), κ constant region (mCκ, solid rectangle), and targeted hCκ allele (open rectangle). Left and right panels show expression of mCκ and hCκ in bone marrow immature (B220$^{low}$IgM$^{high}$) and spleen mature (B220$^{high}$IgM$^+$) Igκ$^{m/h}$ B cells, respectively. (**B**) Analysis of splenocytes from IgH$^{B1-8/+}$-Igκ$^{αHEL/h}$, IgH$^{3H9/+}$Igκ$^{Vκ8/h}$, IgH$^{3H9/+}$Igκ$^{Vκ4/h}$, IgH$^{B1-8high/+}$Igκ$^{αHEL/h}$, and IgH$^{B1-8low/+}$ Igκ$^{αHEL/h}$ mice. The configuration of the Ig heavy (IgH) and Ig κ chain (Igκ) loci is indicated at the top of each column. Antibodies used for staining were anti–B220 conjugated with allophycocyanin (APC), anti–IgM conjugated with biotin [revealed with SA-RED613 (Gibco BRL)], rat antibody to human κ conjugated with fluorescein isothiocyanate (FITC), rat antibody to mouse κ conjugated with phycoerythrin (PE), and anti-Igλ conjugated with PE. Black stripes inside B1–8$^{high}$ and B1–8$^{low}$ genes depict mutations. Dot plots in the second row show cells pregated on B220$^+$; numbers indicate percentage of B220$^+$ cells.

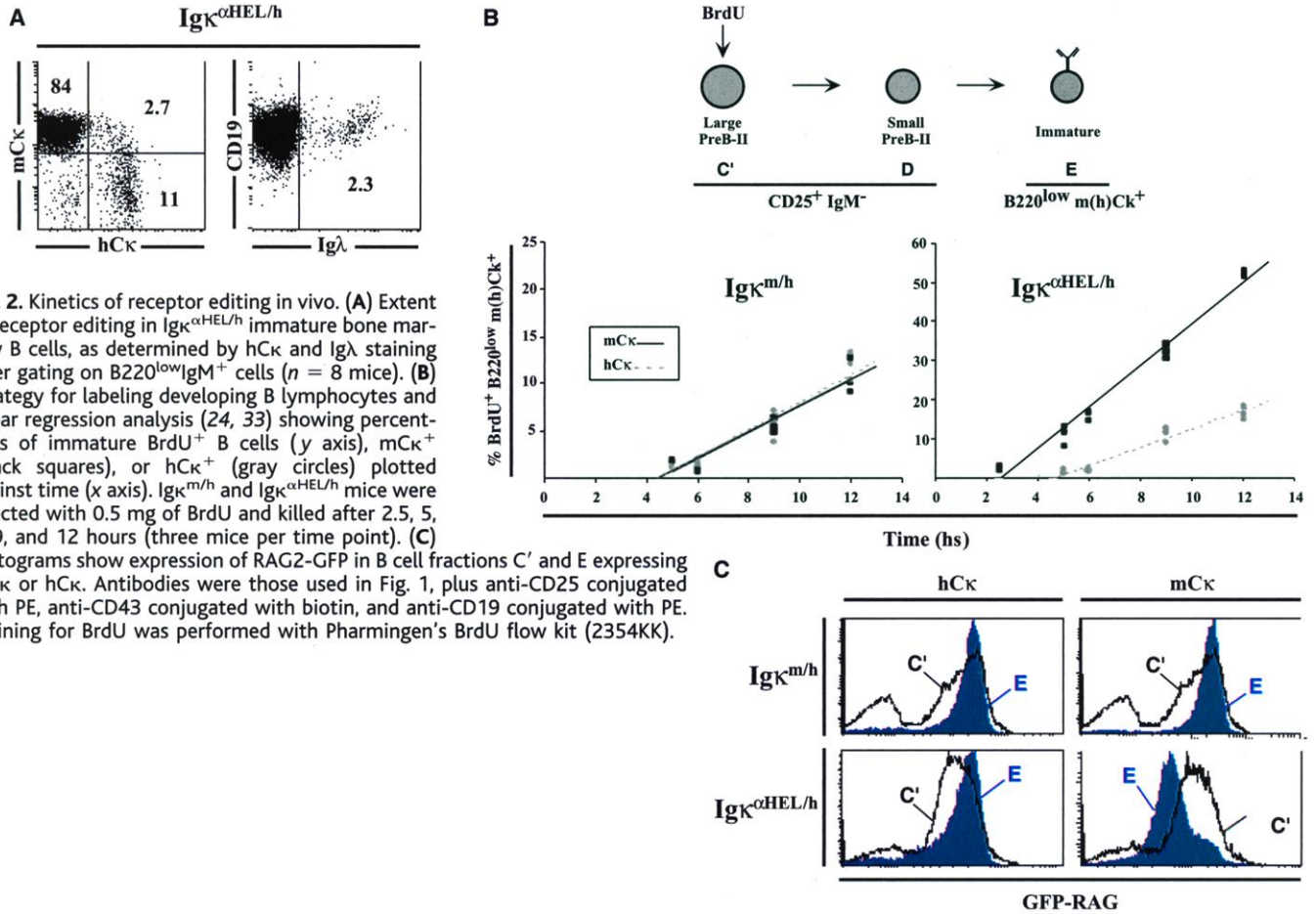



**Fig. 2.** Kinetics of receptor editing in vivo. (**A**) Extent of receptor editing in Igκ$^{\alpha HEL/h}$ immature bone marrow B cells, as determined by hCκ and Igλ staining after gating on B220$^{low}$IgM$^{+}$ cells ($n$ = 8 mice). (**B**) Strategy for labeling developing B lymphocytes and linear regression analysis (*24, 33*) showing percentages of immature BrdU$^{+}$ B cells ($y$ axis), mCκ$^{+}$ (black squares), or hCκ$^{+}$ (gray circles) plotted against time ($x$ axis). Igκ$^{m/h}$ and Igκ$^{\alpha HEL/h}$ mice were injected with 0.5 mg of BrdU and killed after 2.5, 5, 6, 9, and 12 hours (three mice per time point). (**C**) Histograms show expression of RAG2-GFP in B cell fractions C′ and E expressing mCκ or hCκ. Antibodies were those used in Fig. 1, plus anti-CD25 conjugated with PE, anti-CD43 conjugated with biotin, and anti-CD19 conjugated with PE. Staining for BrdU was performed with Pharmingen's BrdU flow kit (2354KK).

clude that editing is not the result of random premature recombination in pro-B cells followed by selection at later stages. Instead, editing is induced in specific B cells during a 2-hour developmental delay at a stage when they are normally recombining their light chain genes. The observation that cells carrying "perfect" receptors [for instance, IgH$^{B1-8/+}$Igκ$^{\alpha HEL/h}$ (Fig. 1B) (*8*)] do not undergo editing further reinforces this conclusion.

To examine RAG expression in B cells undergoing editing in vivo, we combined the Igκ$^{\alpha HEL/h}$ allele with a RAG2-GFP indicator transgene (*25*). Like RAG2 expression, GFP (green fluorescent protein) expression is first induced in pro-B cells undergoing heavy chain recombination, and it decreases in large cycling pre-BII cells; GFP and RAG are normally reinduced in small pre-BII cells undergoing light chain gene rearrangements, and GFP remains elevated in immature B cells (*25, 26*). Consistent with their rapid transit through the small pre-BII stage, unedited B cells (mCκ$^{+}$) from Igκ$^{\alpha HEL/h}$ mice failed to reinduce RAG2; in contrast, edited cells showed high levels of RAG2-GFP expression (Fig. 2C). Thus, B cells expressing functional receptors proceed rapidly to the immature B cell stage, where RAG expression and recombination are down-regulated (*25, 26*). In contrast, B cells expressing receptors targeted for editing are arrested in the RAG$^{+}$ pre-BII compartment. B cells that fail to edit in this compartment are likely to undergo negative selection (*8, 27–29*).

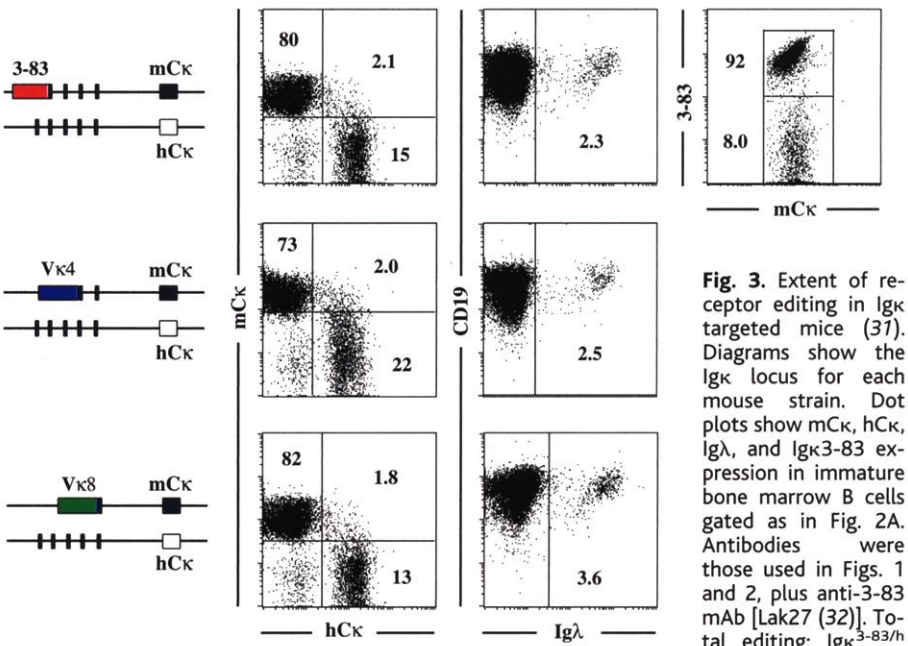



**Fig. 3.** Extent of receptor editing in Igκ targeted mice (*31*). Diagrams show the Igκ locus for each mouse strain. Dot plots show mCκ, hCκ, Igλ, and Igκ3-83 expression in immature bone marrow B cells gated as in Fig. 2A. Antibodies were those used in Figs. 1 and 2, plus anti-3-83 mAb [Lak27 (*32*)]. Total editing: Igκ$^{3-83/h}$ mice ($n$ = 6), 24% (±3); Igκ$^{Vκ4/h}$ mice ($n$ = 8), 33% (±3); and Igκ$^{Vκ8/h}$ mice ($n$ = 6), 18% (±1).

Current models of receptor editing favor a return of self-reactive immature B cells to the pre-BII stage (*29*). Our observations indicate that the BCR regulates receptor editing by controlling the rate of B cell development. B cells with self-reactive antibodies and those cells that have not yet expressed a receptor are delayed in the $RAG^+$ small pre-BII cell compartment, where normal light chain rearrangement takes place. B cells expressing innocuous receptors transit rapidly from this stage to the immature compartment, where RAG gene expression and V(D)J recombination are down-regulated (*25, 26*). This new model clarifies how allelic exclusion is maintained in B cells despite high levels of receptor editing: B cells that deposit non–self-reactive antibodies on their cell surface rapidly turn off V(D)J recombination.

To estimate the extent of receptor replacement normally occurring in vivo, we combined the hCκ allele with three additional pre-rearranged Vκ-Jκ genes: 3-83κi, Vκ4R, and Vκ8R (*30, 31*). B cells that undergo receptor editing and replace the original mouse allele were enumerated by flow cytometry and mRNA analysis. Among these, $Igκ^{3\text{-}83/h}$ mice are unique in that replacement of the Vκ3-83 gene can be detected by loss of staining with a monoclonal antibody specific for Vκ3-83 (*32*). We found that about 25% of the B cells in $Igκ^{3\text{-}83/h}$ mice and 33% of the B cells in $Igκ^{Vκ4/h}$ mice substituted their light chains during B cell development (Fig. 3 and Table 1). $Igκ^{Vκ8/h}$ mice can only delete Vκ8R by RS recombination; nevertheless, 18% (±1) of B cells in these mice replaced the targeted gene and expressed hCκ or Igλ on the cell surface [Fig. 3 (*31*)]. Despite differences in the level of Ig κ chain editing, the amount of Igλ expression was similar in all strains (Figs. 2A and 3 and Table 1).

Our data from four separate Igκ knock-in mouse strains show that about 25% (±7) of the light chains found on the surface of developing B cells in vivo are produced by receptor editing. Whether all of these replacements are induced by self-reactivity is currently unknown. Nevertheless, extrapolating from these experiments, we conclude that receptor editing makes an important contribution to the normal antibody repertoire.

**References and Notes**


1. M. F. Burnet, *The Clonal Selection Theory of Acquired Immunity* (Cambridge Univ. Press, Cambridge, 1959).
2. D. Gay, T. Saunders, S. Camper, M. Weigert, *J. Exp. Med.* **177**, 999 (1993).
3. C. C. Goodnow *et al.*, *Nature* **334**, 676 (1988).
4. D. A. Nemazee, K. Burki, *Nature* **337**, 562 (1989).
5. M. Okamoto *et al.*, *J. Exp. Med.* **175**, 71 (1992).
6. S. L. Tiegs, D. M. Russell, D. Nemazee, *J. Exp. Med.* **177**, 1009 (1993).
7. C. Chen, E. L. Prak, M. Weigert, *Immunity* **6**, 97 (1997).
8. R. Pelanda *et al.*, *Immunity* **7**, 765 (1997).
9. C. Coleclough, R. P. Perry, K. Karjalainen, M. Weigert, *Nature* **290**, 372 (1981).
10. M. W. Retter, D. Nemazee, *J. Exp. Med.* **188**, 1231 (1998).
11. T. Yamagami, E. ten Boekel, J. Andersson, A. Rolink, F. Melchers, *Immunity* **11**, 317 (1999).
12. Supplemental material is available at *Science* Online (www.sciencemag.org/cgi/content/full/291/5508/1541/DC1).
13. E. Sonoda *et al.*, *Immunity* **6**, 225 (1997).
14. $B220^+mCκ^+$ cells were isolated by cell sorting using a FACSVantage instrument (Beckton Dickinson) to greater than 99% purity. cDNA was synthesized from total RNA using random hexamers and SuperScript II (Gibco BRL). Vκ-Jκs were then amplified in a reverse transcription polymerase chain reaction with the following primers: 3′ primer mCκ: ACGCCATTTTGTCGTTCACTGCCA (for $Igκ^{m/h}$, $IgH^{3H9/+}Igκ^{Vκ4/h}$, $Igκ^{αHEL/h}$, and $Igκ^{Vκ4/h}$ mice) or hCκ: GAGTTACCCGATTGGAGGGCGTTA ($Igκ^{m/h}$) and a 5′ universal primer.
15. D. Allen, T. Simon, F. Sablitzky, K. Rajewsky, A. Cumano, *EMBO J.* **7**, 1995 (1988).
16. M. Hertz, D. Nemazee, *Immunity* **6**, 429 (1997).
17. U. Braun, K. Rajewsky, R. Pelanda, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7429 (2000).
18. V. Kouskoff, G. Lacaud, K. Pape, M. Retter, D. Nemazee, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7435 (2000).
19. T. I. Novobrantseva *et al.*, *J. Exp. Med.* **189**, 75 (1999).
20. D. Opstelten, D. G. Osmond, *J. Immunol.* **131**, 2635 (1983).
21. R. R. Hardy, C. E. Carmack, S. A. Shinton, J. D. Kemp, K. Hayakawa, *J. Exp. Med.* **173**, 1213 (1991).
22. D. M. Allman, S. E. Ferguson, V. M. Lentz, M. P. Cancro, *J. Immunol.* **151**, 4431 (1993).
23. B. Rocha *et al.*, *Eur. J. Immunol.* **20**, 1697 (1990).
24. Data from $Igκ^{h/m}$ and $Igκ^{αHEL/h}$ mice were subjected to linear regression analysis, and the entry point for each of the populations was calculated on the basis of the regression formula $y = Bx - A$. The equations obtained were as follows: $Igκ^{h/m}$ hCκ population, $y = 1.44x - 6.33$, entry point [4.4, 0]; mCκ population, $y = 1.40x - 6.35$, entry point [4.5, 0]; $Igκ^{αHEL/h}$ hCκ population, $y = 2.34x - 10.8$, entry point [4.61, 0]; mCκ population, $y = 5.29x - 13.6$, entry point [2.57, 0]. Three mice were killed for each time point.
25. W. Yu *et al.*, *Science* **285**, 1080 (1999).
26. U. Grawunder *et al.*, *Immunity* **3**, 601 (1995).
27. C. Chen *et al.*, *Nature* **373**, 252 (1995).
28. S. B. Harley *et al.*, *Cell* **72**, 325 (1993).
29. D. Melamed, D. Nemazee, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9267 (1997).
30. R. Pelanda, S. Schaal, R. M. Torres, K. Rajewsky, *Immunity* **5**, 229 (1996).
31. E. L. Prak, M. Weigert, *J. Exp. Med.* **182**, 541 (1995).
32. Lak27 (anti-3-83) was raised in an Igκ-deficient mouse immunized with the 3-83 antibody.
33. The mCκ cell population from $Igκ^{αHEL/h}$ mice consists of unedited VκαHEL expressors, as well as a small number of cells (8.5%, Table 1) that have edited the targeted light chain on the mCκ allele. This population would not be seen in our assay. Although not shown in Fig. 2, $BrdU^+λ^+$ cells appeared in the immature compartment after 24 hours of BrdU injection [H. Arakawa, T. Shimizu, S. Takeda, *Int. Immunol.* **8**, 91 (1996)].


34. We thank M. Weigert for $Igκ^{Vκ4}$ and $Igκ^{Vκ8}$ mice, and members of the Nussenzweig lab for suggestions. Supported by grants from Deutsche Forschungsgemeinschaft through SFB 243 (K.R.), by NIH grant 33890 (M.C.N.), and by an NSF predoctoral fellowship (R.C.). M.C.N. is a Howard Hughes Medical Institute investigator.

16 October 2000; accepted 4 January 2001


# Induction of Direct Antimicrobial Activity Through Mammalian Toll-Like Receptors


**Sybille Thoma-Uszynski,[1]* Steffen Stenger,[2]* Osamu Takeuchi,[3] Maria Teresa Ochoa,[1] Matthias Engele,[2] Peter A. Sieling,[1] Peter F. Barnes,[4] Martin Röllinghoff,[2] Pal L. Bölcskei,[5] Manfred Wagner,[5] Shizuo Akira,[3] Michael V. Norgard,[6] John T. Belisle,[7] Paul J. Godowski,[8] Barry R. Bloom,[9] Robert L. Modlin[1]†**



**The mammalian innate immune system retains from *Drosophila* a family of homologous Toll-like receptors (TLRs) that mediate responses to microbial ligands. Here, we show that TLR2 activation leads to killing of intracellular *Mycobacterium tuberculosis* in both mouse and human macrophages, through distinct mechanisms. In mouse macrophages, bacterial lipoprotein activation of TLR2 leads to a nitric oxide–dependent killing of intracellular tubercle bacilli, but in human monocytes and alveolar macrophages, this pathway was nitric oxide–independent. Thus, mammalian TLRs respond (as *Drosophila* Toll receptors do) to microbial ligands and also have the ability to activate antimicrobial effector pathways at the site of infection.**


The primitive immune system of *Drosophila* has evolved to be highly efficient at combating microbial pathogens, largely through a family of cell-surface receptors known as Toll. The activation of Toll proteins by microbial ligands triggers an intracellular signaling pathway involving nuclear factor κB (NF-κB) homologs that leads to the transcription of genes encoding antimicrobial proteins (*1–3*). The *Drosophila* Toll system is structurally conserved and homologous to the mammalian TLR family (*4*). Microbial ligands, including lipopolysaccharide (LPS) and bacterial lipoproteins, have been shown to activate mammalian TLRs, facilitating transcription of genes that regulate the adaptive response, including cytokines and costimulatory molecules (*4–10*). It remains unclear, however, whether activation of mammalian TLRs triggers direct antimicrobial effector pathways.