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Contribution of Receptor Editing to the Antibody Repertoire

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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-B1 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 tolerated were subsequently uncovered using transgenic mice carrying antibody genes coding for self-reactive antibodies, and subsequently uncovered using transgenic mice (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, Igκm/h (12)]. Igκm/h mice showed 43% hCκ, 44% mCκ, and 5% double-expressing B cells by flow cytometry (Fig. 1A). Individual double producers were isolated and their Igκ genes amplified and sequenced, we found that only 1.5% of B cells in Igκm/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 Igκm/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 (IgH3H9/Igκv4/h) or single-stranded (ss) DNA (IgH3H9/Igκv8/h), or an antibody with no apparent self-reactivity (IgH1–8/k/IgκHEL/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 IgH1–8/k/IgκHEL/h and IgH1–8/k/Igκv8/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 IgH3H9/Igκv4/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 IgH3H9/Igκv4/h mice expressed Igκ. We conclude that expression of hCκ correlates with previous measurements of receptor editing.

The extent of editing of IgHκ antibodies depends on the light chain because B cells expressing IgHκκ1κVκ4 antibodies are not edited, whereas B cells expressing IgHκκ4κVκκ4 antibodies are nearly entirely edited.

To investigate whether receptor editing in Igκm/h mice is induced by changes in the antibody combining site, we generated mice carrying two IgH1–8 variants detected during the immune response to 4-hydroxy-3-nitrophénylactyl (NP) (15). Replacement of Trp13 by Leu in complementarity-determining region CDR1 of IgH1–8 increases the affinity for NP by a factor of 10 (IgH1–8), whereas the four amino acid changes found in hybridoma 3C52 decrease NP binding by a factor of 4 (IgH1–8low) (15). Each of these naturally occurring mutations, in combination with the κHEL heavy 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 IgH1–8shigh+IgκHEL/h mice and 64% of B cells in IgH1–8low+hCκHEL/h mice expressed hCκ on their cell surface. Increased Igκ usage in the mouse B cell repertoire has often been used as a marker for receptor editing (6, 10, 16). However, increased Igκ expression was only seen in IgH1–8shigh mice (Fig. 1B). The low level of surface Igκ expression in IgH1–8low B cells is not due to the inability of this heavy chain to pair with a light chain, because IgH1–8low Igκ is a naturally occurring antibody combination (15). Thus, surface Igκ 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 IgH1–8 targeted genes is identical, different Igκ 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 κHEL light chain (12). Thus, it appears that the B1–8high and B1–8low heavy chains produce self-reactive antibodies when combined with VκαHEL. We conclude that Igκm/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, Igκ heavy chain gene recombination is not constrained. Therefore, VκαHEL light chains, expressed at the pre-B1 cell stage, are paired with the full spectrum of mouse heavy chains, an unknown number of which are autoreactive.

Table 1. Receptor editing on targeting alleles. mCκ+ cells from IgH3H9/Igκv4/h, IgH3H9/IgκHEL/h, and IgH3H9/Igκv4/h mice were sorted and their Vκ-Jκ (mCκ) genes isolated and sequenced.

<table>
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Fig. 1. Targeting hCk. (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<sup>hi</sup> IgM<sup>hi</sup>) and spleen mature (B220<sup>lo</sup> IgM<sup>lo</sup>) B cells, respectively. (B) Analysis of splenocytes from Igκ<sup>ΔB1-Δκκ</sup> αHEL-expressing B cells. We found that VκαHEL light chains are frequently replaced during B cell development. Analysis of bone marrow Igκ<sup>H-1/L-1</sup> 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 mlg mRNAs from sorted mCκ<sup>+</sup> B cells. We found that 8.5% of the Igκ mRNAs from purified mCκ<sup>+</sup> lymphocytes were products of receptor editing (6% of the B cells, Table 1). Therefore, about 22% of newly formed B cells in Igκ<sup>H-1/L-1</sup> 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-κκ recombination in pre-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<sup>HEL</sup>-<sup>h</sup> 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 (Igκ<sup>+</sup>) (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<sup>HEL</sup> 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<sup>+</sup>) emerged 2.6 hours after BrdU injection in Igκ<sup>HEL</sup> 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<sub>2</sub> 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<sup>+</sup>), all of the edited B cells from the same mice (hCκ<sup>+</sup>) 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<sup>Δκκ</sup>/<sup>Δκκ</sup> and Igκ<sup>κκ</sup>κκ/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-
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, IgHβ/Igk hel/h (Fig. 1B) (8)] do not undergo editing fur-ther reinforces this conclusion.

To examine RAG expression in B cells undergoing editing in vivo, we combined the Igk 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 (mCc) from Igk 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 recom-bination 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. 2. Kinetics of receptor editing in vivo. (A) Extent of receptor editing in Igk hel/h immature bone marrow B cells, as determined by hCx and Igk staining after gating on B220low 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), mCc+ (black squares), or hCx+ (gray circles) plotted against time (x axis). Igk+m/h and Igk 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 mCc or hCx. 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).

Fig. 3. Extent of receptor editing in Igk targeted mice (37). Diagrams show the Igk locus for each mouse strain. Dot plots show mCc, hCx, Igk, and Igk Vκ4 expres-sion 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: Igk 3-83/h mice (n = 6), 24% (±3); Igk Vκ4/h mice (n = 8), 33% (±3); and Igk Vκ8/h mice (n = 6), 18% (±1).
Current models of receptor editing favor a return of self-reactive immature B cells to the pre-B1I 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-2 small pre-B1I 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: 3-83κ, 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, Igκ8R-hCκ 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 Igκ3-83κ/hCκ mice and 33% of the B cells in Igκ8R-hCκ mice substituted their light chains during B cell development (Fig. 3 and Table 1). Igκ8R-hCκ 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 Igκ on the cell surface [Fig. 3 (31)].

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


Induction of Direct Antimicrobial Activity Through Mammalian Toll-Like Receptors

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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 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-kappa B (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 co-stimulatory (5–10). It remains unclear, however, whether activation of mammalian TLRs triggers direct antimicrobial effector pathways.