Human Autoantibody Silencing by Immunoglobulin Light Chains

Hedda Wardemann,1 Johanna Hammersen,1 and Michel C. Nussenzweig1,2

1Laboratory of Molecular Immunology, The Rockefeller University and 2Howard Hughes Medical Institute, New York, NY 10021

Abstract

Several newly arising human antibodies are polyreactive, but in normal individuals the majority of these potentially autodestructive antibodies are removed from the repertoire by receptor editing or B cell deletion in the bone marrow. To determine what proportion of naturally arising autoantibodies can be silenced by immunoglobulin (Ig) light chain receptor editing, we replaced the light chains in 12 such antibodies with a panel of representative Igκ and Igλ chains. We found that most naturally arising autoantibodies are readily silenced by light chain exchange. Thus, receptor editing may account for most autoreactive antibody silencing in humans. Light chain complementarity determining region (CDR) isoelectric points did not correlate with silencing activity, but Igκ genes were more effective than Igλ genes as silencers. The greater efficacy of Igλ chains as silencers of autoreactivity provides a possible explanation for the expansion and altered configuration of the Igλ locus in evolution.

Key words: human • tolerance • B lymphocytes • receptor editing • autoantibodies

Introduction

Antibody genes are assembled by random recombination of Ig variable (V), diversity (D), and joining (J) gene segments leading to the production of diverse antibody repertoire (1). Diversity is essential to ensure that the immune system can recognize several potential pathogens, but the cost of producing receptors by random gene recombination is that many antibodies are self-reactive (2). In mice, the majority of these potentially self-destructive autoantibodies are removed from the repertoire in the early stages of B cell development by receptor editing or deletion, and the few self-reactive B cells that escape central censorship are rendered anergic (3–7). Although the extent of autoreactive B cell deletion and anergy is unknown, it has been estimated that in the mouse, receptor editing is an important contributor to the antibody repertoire, accounting for 25–50% of all antibodies (8–10).

Receptor editing is fundamentally different from deletion or anergy in that it spares self-reactive B cells by producing nonself-reactive receptors (3–5). Both Ig heavy (IgH) and Ig light (IgL) chains can be replaced by editing, but light chain replacement appears to be the dominant form of receptor editing (3–5, 11–28).

Receptor editing has been studied primarily in mice carrying transgenes that encode somatically mutated antibodies derived from autoimmune MRL/lpr (anti-DNA, 3H9) or immunized (anti-MHC, 3-83) mice (3–5, 11–29). The molecular basis for silencing DNA-binding by 3H9 IgH chain is neutralization of positively charged IgH chain complementarity determining region (CDR) arginine residues by light chains that have negatively charged CDRs (16, 25, 28). Based on these observations, it was proposed that anti-DNA editor light chains have low CDR isoelectric points (pIs) with aspartate residues at key positions (16, 25, 28). The editor light chains for the anti-MHC antibody 3-83 have not been defined, but 3-83 editing is associated with increased Igλ expression, suggesting that Igλ contributes to receptor editing (4, 8, 19, 21, 22, 30). Further evidence for a role of Igλ in receptor editing in mice comes from the observation that 47% of all Igλ-expressing B cells carry productively rearranged Igκ genes (8). However, not all Igλ genes in the mouse serve as editors, and in some cases, Igλ appears to increase self-reactivity (9, 12, 14, 26, 27).

The majority of the antibodies produced by early immature B cells are autoreactive (2), but little is known about receptor editing of naturally arising autoantibodies (28). To
examine the IgL chain features that regulate silencing of naturally arising autoantibodies in humans, we systematically exchanged the IgL chains cloned from such antibodies with a collection of 12 selected Igκ and Igλ chains. Here, we report that most of the self-reactive antibodies normally generated in human bone marrow can be silenced by IgL chain replacement and that human Igκ light chains are more effective silencers than Igλ chains.

Materials and Methods

Antibody Production and Purification. Heavy and light chain cDNAs were the same as reported previously (2). For antibody production, 293A human embryonic kidney fibroblasts were transfected as described previously (2). In brief, cells were cultured in DMEM supplemented with 10% ultra-low IgG FCS (GIBCO BRL) and cotransfected with IgH and IgL chain–encoding plasmid DNA by calcium phosphate precipitation. 8–12 h after transfection, cells were washed with serum-free DMEM and thereafter cultured in DMEM supplemented with 1% Nutridoma SP (Roche). Supernatants were collected after 8 d of culture. Antibodies were purified by binding to protein G–Sepharose™ (Amersham Biosciences) and eluted with 0.1 M glycine buffer (pH 3). Antibody concentrations in tissue culture supernatants and after purification were determined by anti–human IgG1 ELISA using human monoclonal IgG1 as standard (Sigma-Aldrich).

ELISAs. ELISAs were performed as described previously (2). In brief, tissue culture supernatants were adjusted to a starting antibody concentration of 1 μg/ml for polyreactivity ELISAs and used at three subsequent 1:4 dilutions in PBS. Specific antigens were coated on microtiter plates (COSTAR Easywash Polystyrene Plates; Corning) at 10 μg/ml for dsDNA, dsDNA, and LPS (Sigma–Aldrich) or 5 μg/ml for recombinant human insulin (Fitzgerald). Samples were considered negative if the OD at 580 did not exceed a preset threshold value at any of the four dilutions (OD at 580: ssDNA, 0.4; dsDNA, 0.42; insulin, 0.5; and LPS, 0.45) in at least two independent experiments. In all experiments, ED38 (2, 31) was included as positive control and m-GO13 (2) as negative control. HEp–2 ELISAs were performed on QUANTA Lite™ antinuclear antibody (ANA) ELISA plates (INOVA Diagnostics) coated with HEP–2 cell lysates. Purified antibodies were used at a concentration of 25 μg/ml with three subsequent 1:4 dilutions in PBS. The threshold OD at 580 below which samples were considered negative was 0.4. Positive and negative controls included sera from patients and healthy individuals (INOVA Diagnostics) as well as ED38 and were included in every experiment. All ELISAs were developed with horseradish peroxidase–labeled goat anti–human IgG Fc Ab (Jackson ImmunoResearch

Figure 1. Characteristics of selected polyreactive antibodies, Igκ and Igλ light chains. (A) ELISA reactivity of antibodies from early immature B cells with ssDNA, dsDNA, insulin, and LPS (reference 2). Positive control antibody ED38 (references 2, 31) is shown as a dashed line. (B) CDR plS and CDR3 sequences of the Igκ and Igλ light chains used for IgL chain exchange experiments. *the Vκ1-5 gene used in these experiments has a CDR pl of 8.9 due to a polymorphism in CDR2 (Table S1). (C) Relative representation of individual human Vκ and VA genes in the repertoire of expressed human antibodies (reference 2). V genes are ordered 5’ to 3’ according to their position in the chromosome relative to the IgL chain constant region genes. (D) CDR plS of functional human Igκ (○) and Igλ (●) V genes. Arrows indicate V genes selected for Igκ chain exchange experiments except for Vκ1-5 as indicated (*), for which a polymorphic version of the gene with a CDR pl of 8.9 was used (B). CDR plS are displayed on the y axis.
Laboratories) and horseradish peroxidase substrate (Bio-Rad Laboratories). OD₄₅₀ was measured on a microplate reader (Molecular Devices).

Indirect Immunofluorescence Assay (IFA). IIFAs were performed as described previously (2). In brief, HEp-2 cell coated slides (Bion Enterprises, Ltd.) were incubated at room temperature with purified antibodies at 25–150 μg/ml for 30 min, washed in PBS, and visualized with FITC anti–human Ig by fluorescence microscopy. Controls included ED38, and positive and negative sera (Bion Enterprises, Ltd.).

Calculation of pIs. CDR pIs were calculated based on CDR1, CDR2, and CDR3 amino acid sequences.

Statistics. p-values were calculated by two-tailed Fisher Exact test.

Online Supplemental Material. Table S1 shows heavy and light chain Ig gene repertoire and ELISA reactivity of antibodies from which individual IgH and IgL chains were derived. Table S2 depicts ELISA reactivity with ssDNA, dsDNA, LPS, and insulin of antibodies expressing IgH chains (ei17, ei62, ei33, and ei102) from early immature B cells that cannot be silenced by IgL chain receptor editing. Table S3 shows ELISA reactivity with ssDNA, dsDNA, LPS, insulin, and HEp-2 and HEp-2 IFA of antibodies expressing IgH chains (ei95, ei40, ei141, ei69, and ei115) from early immature B cells that can be silenced by IgL chain receptor editing. Table S4 depicts ELISA reactivity with ssDNA, dsDNA, LPS, and insulin of antibodies expressing IgH chains from new emigrant (ne5, ne77) and mature (m37) B cells that can be silenced by IgL chain receptor editing. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20040818/DC1.

Results

Many naturally arising autoantibodies react with several different self-antigens including DNA and are referred to as polyreactive. These antibodies make up a majority of all newly arising antibodies in humans. However, few mature B cells produce polyreactive antibodies because they are removed from the repertoire during B cell development in the bone marrow, primarily in the transition between the early immature and the immature B cell stage. This stage in B cell development corresponds to the stage associated with receptor editing and autoreactive B cell deletion.

Silencing Polyreactive Antibodies. To determine whether naturally arising human autoreactive antibodies can be silenced by IgL chain replacement, we coexpressed IgH chains from nine polyreactive early immature B cell antibodies with a panel of different IgL chains (2). The polyreactive antibodies selected were representative of such antibodies in that they showed varying affinities for ssDNA, dsDNA, insulin, and LPS (Fig. 1 A and reference 2). Three highly polyreactive heavy chains (ei17, ei33, and ei62), representing 10% of the initial antibody repertoire, were selected, whereas the other six (ei40, ei69, ei95, ei102, ei115,
and ei141), representing 45% of the initial repertoire, were less polyreactive (2). Each of the nine heavy chains was paired with each of six Igκ or six Igλ chains that had been cloned from B cells from the same healthy donors (Fig. 1 B, Table S1, available at http://www.jem.org/cgi/content/full/jem.20040818/DC1, and reference 2). These IgL chains were selected based on frequency of V gene usage and the range of IgL chain CDR pIs (Fig. 1 B–D, and reference 2). The IgL chain panel includes 10 of the most frequently used human Igκ and Igλ V genes as well as Vk1-33, with a CDR pl of 3.1, and VA7-46, with a CDR pl of 8.7 (Fig. 1 B–D). Together, the selected V regions of the IgL chains cover 63% of the Vk and 61% of the Vλ genes found in the normal human antibody repertoire (2, 32, 33) and their CDR pIs range from 3.1 to 10.9 (Fig. 1 B–D).

Nearly all combinations of heavy and light chains (129 out of 144) were efficiently produced in transfected tissue culture cells (≥1 μg/ml of supernatant), suggesting that the majority of IgH chains expressed by human B cells are compatible with a wide range of light chains (Tables S1–S4, available at http://www.jem.org/cgi/content/full/jem.20040818/DC1). We found that four of the nine IgH chains (ei17, ei33, ei62, and ei102) could not be silenced by any of the κ- or λ-IgL chains tested (Fig. 2 and Table S2). Three of these “nonsilenceable” IgH chains (ei17, ei62, and ei33) were cloned from rare early immature B cells expressing highly reactive antibodies (10% of all polyreactive antibodies expressed in early immature B cells; reference 2). In addition, IgH ei33 was originally cloned from an unusual B cell that expressed two IgL chains, one Igκ and one Igλ (Figs. 1 A and 2). The reactivity of these heavy chains was barely modulated by any of the IgL chains, including Vk1-33 and Vλ1-44 with CDR pIs of 3.1 and 3.9, respectively. The fourth IgH chain in the nonsilenceable group (ei102) was different from the other three in that the original antibody showed modest levels of polyreactivity and almost all of the light chains tested increased polyreactivity when compared with the original (Fig. 2). The remaining five polyreactive antibodies from immature B cells showed the low to intermediate levels of autoreactivity displayed by 45% of all antibodies expressed in early immature B cells (ei95, ei40, ei141, ei69, and ei115; reference 2). These antibodies were readily silenced by light chain exchange (Fig. 3 and Table S3). However, each heavy chain displayed a different pattern of silencing by IgL chains, and some IgL chains even enhanced the reactivity of some heavy chains.

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**Figure 3.** Responsive polyreactive antibodies. Data show ELISA reactivity on ssDNA, dsDNA, Insulin, and LPS for ei95, ei40, ei141, ei69, and ei115 IgH chains combined with different IgL chains. The original IgL chain is represented in red, those that failed to silence in black, those that silenced reactivity in green, and the positive control antibody ED38 (references 2, 31) as a dashed line. The horizontal line in each graph shows the threshold for positive reactivity. For each IgH chain, the number of effective editors of polyreactivity as a proportion of the number of light chains tested is indicated (bottom).
In one unusual case, the original light chain might have been responsible for polyreactivity, ei95, because exchange with any Igκ or Igλ silenced the reactivity of this antibody (Fig. 3). We conclude that highly reactive autoantibodies found in 10% of all early immature B cells are difficult to silence, whereas most of the remaining polyreactive antibodies representing ~45% of the repertoire can readily be silenced by light chain exchange.

Silencing ANAs. Polyreactive antibodies are efficiently removed from the repertoire between the early immature and the immature stage of B cell development (2). However, several B cells that pass this first checkpoint still express autoreactive antibodies as measured by HEp-2 cell ELISA and indirect immunofluorescence, which are standard clinical tests for autoantibodies (2). To determine whether light chains that silenced polyreactivity also alter this other form of autoreactivity, we tested four of the silenceable heavy chains (ei40, ei141, ei69, and ei115) in HEp-2 ELISAs (Fig. 3). With one exception, all polyreactive antibodies were also reactive in the HEp-2 cell ELISA (Fig. 4 and Table S3), but silencing polyreactivity did not always correlate with silencing for HEp-2 reactivity (Fig. 4 and Table S3). For example, 9 out of 12 of the IgL chains tested silenced polyreactivity for IgH chain ei40 but only 2 of these completely abolished autoreactivity as measured in the HEp-2 cell ELISA and IFA (Figs. 3 and 4 and Table S3). In contrast, 3 out of 10 IgL chains tested silenced polyreactivity for IgH chain ei69 and all 3 also abolished HEp-2 cell reactivity (Fig. 4 and Table S3). However, light chains that failed to silence HEp-2 cell reactivity modulated the nature of the autoreactivity as measured by indirect immunofluorescence (Fig. 4 B and Table S3). Polyreactive antibodies from early immature B cells frequently show nuclear and cytoplasmic staining by immunofluorescence (Fig. 4 B and reference 2). Pairing with nonnative IgL chains that silenced polyreactivity typically altered the pattern of HEp-2 cellular staining (Fig. 4 B). We conclude that loss of polyreactivity by light chain replacement does not always correlate with silencing HEp-2 cell reactivity. This difference in silencing between polyreactivity and HEp-2 reactivity may explain why large numbers of HEp-2 cell–reactive B cells remain until later stages of B cell development even after polyreactive antibodies are removed from the repertoire (2). Finally, our findings are consistent with the suggestion that light chains contribute to ANA autospecificity (11).

Peripheral Polyreactive Antibodies. Although the majority of polyreactive antibodies are counter-selected between the early immature to immature B cell stage in the bone marrow, a small number of antibodies showing low levels of polyreactivity can be detected in the periphery (2). To determine if these antibodies could be silenced by light chain replacement, we coexpressed each of three such IgH chains (ne5, ne77, and m37) with our panel of Igκ and Igλ chains and tested the antibodies for polyreactivity (Fig. 5). We found that all three of these heavy chains were readily silenced by IgL chain replacement (Fig. 5 and Table S4). We conclude that, in humans, the few peripheral B cells expressing antibodies with low levels of polyreactivity could have been edited in the bone marrow, but were not. Thus, editing is either incomplete or unnecessary for antibodies with low levels of polyreactivity.

pI and Light Chain Isotype. In the mouse, there is a direct correlation between the ability of a light chain to serve as an editor of anti-DNA antibodies and low CDR pIs (25, 28). Similar rules do not appear to apply to silencing of

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naturally arising polyreactive antibodies with anti-DNA reactivity in humans (Fig. 6). We found no correlation between Igκ or Igλ CDR pl and silencing (Fig. 6). For example, Vk1-33 with the lowest CDR pl (3.1) was the only Igκ tested that could not silence DNA binding by IgH chain ei40. In contrast, the same Vk1-33 was the only light chain that was able to silence DNA binding by IgH chain ei115. In addition, Vk3-15 with a CDR pl of 10.9 had the same overall ability to silence DNA binding by nine different heavy chains as Vk1-33 with a CDR pl of 3.1. These observations are consistent with our previous finding that there was no correlation between IgL chain V gene usage and antibody reactivity (Fig. 6, A and B, and reference 2).

Although IgL chain CDR pl did not correlate with silencing, comparison of Igλ and Igκ genes showed that Igλs are more effective than Igκs in antibody silencing (Fig. 6 A, P = 0.01). Individually, all Ig Vλ genes with the exception of VA3-1 were equal to or better silencers than Igκ V genes (Fig. 6 A). To determine whether light chain isotype might influence self-reactivity in vivo, we compared the frequency of Igκ and Igλ in self-reactive and nonself-reactive antibodies found in immature bone marrow B cells and peripheral new emigrant B cells in healthy human donors (2). We found that newly produced self-reactive antibodies were more likely to include Igκ than Igλ light chains (Fig. 6 C, P = 0.001). Although Igκ genes normally recombine before Igλ and, therefore, are unlikely to edit Igλ antibodies, Igκ occasionally silenced Igλ antibodies in vitro. For example, two Igλs and Vk1-33 silenced ei115, a clone originally expressing an Igλ chain. We conclude that, in humans, Igλ light chains are better potential editors of naturally arising polyreactive antibodies than Igκ light chains and that the ability of an IgL chain to silence polyreactivity in humans (Fig. 6).

Figure 5. Polyreactive antibodies cloned from peripheral blood B cells. Data show ELISA reactivity on ssDNA, dsDNA, Insulin, and LPS for ne5, ne77, and ne37 IgH chains combined with different IgL chains. Graphs, symbols, and IgL chain representation as in Fig. 3. For each IgH chain, the number of effective editors of polyreactivity as a proportion of the number of light chains tested is indicated (bottom).

Figure 6. Characteristics of editor light chains. (A) IgL chains that edit individual silenceable IgH chains. IgH chains are indicated (top). Editor IgL chains (left, Igκ; right, Igλ) are shown in black bars above the line, and noneditor IgL chains in white bars are shown below the line. (B) The percentage of all tested IgH chains that could be silenced by a particular Igκ (⊙) or Igλ (●) light chain plotted on the y axis versus CDR pl of the IgL chain on the x axis. (C) The percentage of Igλ (n = 42) or Igκ (n = 83) bearing antibodies cloned from immature and new-emigrant B cells from two healthy donors that were self-reactive (□) or nonself-reactive (●) (reference 2).
activity, including DNA-binding, does not correlate with CDR pI.

Discussion

In humans, random gene V, D, and J segment usage leads to the expression of several autoantibodies in early immature B cells (2). These autoantibodies fall into two groups, polyreactive antibodies and HEp-2 cell binding ANAs. The vast majority of polyreactive antibodies are removed from the repertoire in the transition between the early immature and the immature stage of B cell development. Few polyreactive antibody-producing B cells escape to the periphery and those that do show only low levels of reactivity (2). In contrast, B cells producing HEp-2 cell-reactive ANAs are only partially removed in the early immature to immature B cell transition, and additional selection occurs between the new emigrant and the mature B cell stage in the periphery (2).

Experiments with transgenic mice have established that newly arising self-reactive antibodies are removed by two mechanisms, receptor editing and deletion, and that self-reactive B cells that escape central censorship are rendered anergic (3–7). The mechanism that mediates editing is believed to involve trapping nascent B cells expressing autoantibodies in the early immature stage of B cell development where persistent V(D)J recombination leads to IgV gene replacement. Those B cells that succeed in silencing their self-reactive antibodies by gene replacement are released from the early immature B cell stage and complete B cell development. Several lines of experimental evidence support this kinetic model for receptor editing. For example, there is expansion of the early immature B cell compartment and increased RAG expression in mice carrying transgenic antibodies that are difficult to edit (5, 30, 34), and in the absence of RAG expression, self-reactive B cells are deleted (35). Conversely, self-reactive B cells that are artifically kept alive with Bcl-2 display increased receptor editing (18, 21, 22). Finally, direct measurements show delayed B cell development under conditions of receptor editing (9, 10).

Despite the important contribution of editing to the antibody repertoire, little is known about the ability of light chains to edit naturally arising self-antibodies. The properties of editor light chains have been examined systematically only for the 3H9 anti-DNA antibody, which was derived from a somatically mutated IgG found in the spleen of autoimmune MRL/lpr mice (36). DNA binding by 3H9 is dependent on arginine residues, and only a limited number of Igκ chains with low CDR pIs that neutralize these charges are effective editors (25, 28). The number of light chains that edit the 3H9 IgH chain increases when it is reverted to a lower affinity unmutated germline form. The germline version of 3H9 has reactivity with phosphatidylserine in addition to DNA and, therefore, resembles some of the polyreactive antibodies reported here. Conversely, fewer light chains can edit when IgH chain arginines are added to increase DNA affinity (25, 28). Thus, it was initially surprising to find no apparent correlation between IgL chain CDR pIs and anti-DNA silencing activity in naturally arising human antibodies. However, the mechanism of DNA binding by naturally arising polyreactive antibodies is unknown, and may differ from pathogenic autoantibodies such as 3H9 that are clonally expanded in autoimmune prone mice (36). Long and positively charged IgH CDR3s have been associated with polyreactivity (2, 28, 37). Indeed, 20% of naturally arising human polyreactive antibodies have no positively charged residues in IgH chain CDR3, 27% had a single positive charge, and 53% have two or more positive charges (2). This represents a significant increase in positively charged IgH chain CDR3s in polyreactive antibodies when compared with nonreactive antibodies, but positively charged CDR3s are neither essential for nor diagnostic of polyreactivity in naturally arising antibodies (2).

Polyreactive antibodies frequently show ANA reactivity in clinical ELISA assays on HEp-2 cell lysates. Although polyreactivity is efficiently silenced in the bone marrow, the number of B cells that express autoreactive antibodies as measured by HEp-2 cell ELISA only drops from 76 to 43% between the early immature and immature B cell stages (2). However, the HEp-2 cell-reactive antibodies expressed by immature B cells show preferential loss of nuclear reactivity when compared with those expressed by early immature B cells (2). This is consistent with our observation that polyreactive antibodies silenced by IgL chain exchange frequently retain HEp-2 cell reactivity, but the pattern of staining is altered. Thus, the light chain in these antibodies determines the type of autoreactivity. Preferential loss of polyreactivity by light chain replacement during receptor editing could explain why polyreactivity is efficiently silenced while 43% of immature B cells continue to express HEp-2 cell–reactive ANAs (2).

Light chain exchange silenced most heavy chains with intermediate levels of polyreactivity typical of the majority of early immature polyreactive antibodies, but a subgroup of heavy chains could not be silenced. Three out of the four IgH chains that were refractory to silencing by light chain exchange showed long and charged CDR3s (ε17, ε162, and ε1102). Two of these were initially highly polyreactive (ε17 and ε162), but the third (ε1102) was unusual in that it started off with low-level polyreactivity; all of the IgL chains tested in swapping experiments increased the level of reactivity. This antibody (ε1102) was initially an IgA antibody and, therefore, may have been the end product of receptor editing that could not be further silenced. The fourth nonsilenceable heavy chain (ε33) did not have a long CDR3, but was highly charged and showed the most basic CDR pI of all IgH chains tested (10.1). In addition, IgH chain ε33 was cloned from a cell expressing both Igκ and IgA, a feature associated with extensive receptor editing in the mouse (26). Antibodies with long and charged IgH chain CDR3s represent 10% of the self-reactive antibodies cloned from early immature B cells in normal humans, but
they are rarely found in the periphery (2, 38, 39). If light chain editing is also ineffective against such IgH chains in vivo, then B cells that carry such IgH chains must undergo either heavy chain receptor editing or deletion (13). Consistent with this idea, it has been estimated that 5% of human B cells carry heavy chains that result from receptor editing (40). All of the five remaining antibodies with intermediate levels of polyreactivity representing the majority of polyreactive antibodies found in early immature B cells were silenced. Thus, the majority of naturally arising human self-reactive B cells need not be deleted but can be silenced by IgL chain replacement.

A small number of polyreactive antibodies escape the early immature to immature checkpoint in the bone marrow and can be found in the periphery, and cells producing these antibodies may even be positively selected because they produce “natural antibodies” (2). These antibodies typically show low levels of self-reactivity, and all such antibodies tested were readily silenced by IgL chain exchange. Therefore, editing could have repaired these antibodies, but did not. B cells that express such antibodies (4% of all mature B cells in normal humans) may be anergic. Alternatively, they may be precursors of marginal zone or B1 type cells, which are B cell subpopulations that appear to be enriched in low affinity polyreactive antibodies (41).

In humans, 40% of all antibodies carry IgA light chains, whereas in mice, only 5% are IgA. There are only three Vα genes in the mouse, and the locus is not permissive for receptor editing by nested recombination. In contrast, there are 30 functional Vα genes in humans, and the IgA locus is permissive for receptor editing, but it may not have deletional elements similar to those found in the Igκ locus that limit Igκ receptor editing (42, 43). In humans, B cells expressing IgA invariably have recombined Igκ, but Igκ-expressing cells only occasionally carry recombined Igκ, suggesting an ordered model for light chain gene recombination where Igκ precedes IgA (33, 44, 45). Thus, autoantibodies bearing Igκ and unable to find an Igκ editor would eventually delete the Igκ locus by recombination but could still be silenced by Igκ recombination. Finding that in humans, Igκ is more effective in silencing of naturally arising autoantibodies than Igκ was unexpected, and the structural basis for this difference is not readily apparent, but the observation is consistent with the finding that Igκ-expressing immature and new emigrant B cells are less likely to be self-reactive than Igκ-expressing cells (Fig. 6 C). Given the several self-reactive antibodies produced in early immature B cells, strong selective pressure for editor IgL chains would be expected. Efficient silencing by Igκ provides a potential rationale for the expanded role of the Igκ locus in man.

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References

1. Tonegawa, S. 1983. Somatic generation of antibody diversity. Nature. 302:575–581.
2. Wardemann, H., S. Yurasov, A. Schaefer, J.W. Young, E. Meffre, and M.C. Nussenzweig. 2003. Predominant autoantibody production by early human B cell precursors. Science. 301:1374–1377.
3. Gay, D., T. Saunders, S. Camper, and M. Weigert. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. J. Exp. Med. 177:999–1008.
4. Radic, M.Z., J. Erikson, S. Litwin, and M. Weigert. 1993. B lymphocytes may escape tolerance by revising their antigen receptors. J. Exp. Med. 177:1165–1173.
5. Tzeng, S.L., D.M. Russell, and D. Nemazee. 1993. Receptor editing in self-reactive bone marrow B cells. J. Exp. Med. 177:1009–1020.
6. Nemazee, D.A., and K. Burki. 1989. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. Nature. 337:562–566.
7. Goodnow, C.C., J. Crosbie, S. Adelstein, T.B. Lavoie, S.J. Smith-Gill, R.A. Brink, H. Pritchard-Briscoe, J.S. Wotherpoon, R.H. Loblay, K. Raphael, et al. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature. 334:676–682.
8. Retter, M.W., and D. Nemazee. 1998. Receptor editing occurs frequently during normal B cell development. J. Exp. Med. 188:1231–1238.
9. Casellas, R., T.A. Shih, M. Kleinnweitfeld, J. Rakonjac, D. Nemazee, K. Rajewsky, and M.C. Nussenzweig. 2001. Contribution of receptor editing to the antibody repertoire. Science. 291:1541–1544.
10. Oberdoerffer, P., T.I. Novobrantseva, and K. Rajewsky. 2003. Expression of a targeted A1 light chain gene is developmentally regulated and independent of Igκ rearrangements. J. Exp. Med. 197:1165–1172.
11. Radic, M.Z., M.A. Mascelli, J. Erikson, H. Shan, and M. Weigert. 1991. Ig H and L chain contributions to autoimmune specificities. J. Immunol. 146:176–182.
12. Chen, C., M.Z. Radic, J. Erikson, S.A. Camper, S. Litwin, R.R. Hardy, and M. Weigert. 1994. Deletion and editing of B cells that express antibodies to DNA. J. Immunol. 152:1970–1982.
13. Chen, C., Z. Nagy, E.L. Prak, and M. Weigert. 1995. Immunoglobulin heavy chain gene replacement: a mechanism of receptor editing. Immunity. 3:747–755.
14. Prak, E.L., M. Trounstine, D. Huszar, and M. Weigert. 1994. Light chain editing in κ-deficient animals: a potential mechanism of B cell tolerance. J. Exp. Med. 180:1805–1815.
15. Prak, E.L., and M. Weigert. 1995. Light chain replacement: a new model for antibody gene rearrangement. J. Exp. Med. 182:541–548.
16. Ibrahim, S.M., M. Weigert, C. Basu, J. Erikson, and M.Z. Radic. 1995. Light chain contribution to specificity in anti-DNA antibodies. J. Immunol. 155:3223–3233.
17. Chen, C., E.L. Prak, and M. Weigert. 1997. Editing disease-associated autoantibodies. Immunity. 6:97–105.
18. Lang, J., B. Arnold, G. Hammerling, A.W. Harris, S. Korsmeyer, D. Russell, A. Strasser, and D. Nemazee. 1997. En-
forced Bcl-2 expression inhibits antigen-mediated clonal elimination of peripheral B cells in an antigen dose-dependent manner and promotes receptor editing in autoreactive, immature B cells. *J. Exp. Med.* 186:1513–1522.

19. Pelanda, R., S. Schwers, E. Sonoda, R.M. Torres, D. Nemazee, and K. Rajewsky. 1997. Receptor editing in a transgenic mouse model: site, efficiency, and role in B cell tolerance and antibody diversification. *Immunity.* 7:765–775.

20. Lam, K.P., and K. Rajewsky. 1998. Rapid elimination of mature autoreactive B cells demonstrated by Cre-induced change in B cell antigen receptor specificity in vivo. *Proc. Natl. Acad. Sci. USA.* 95:13171–13175.

21. Melamed, D., and D. Nemazee. 1997. Self-antigen does not accelerate immature B cell apoptosis, but stimulates receptor editing as a consequence of developmental arrest. *Proc. Natl. Acad. Sci. USA.* 94:9267–9272.

22. Melamed, D., R.J. Benshop, J.C. Cambier, and D. Nemazee. 1998. Developmental regulation of B lymphocyte immunoglobulin repertoire compartmentalizes selection from receptor selection. *Cell.* 92:173–182.

23. Xu, H., H. Li, E. Suri-Payer, R.R. Hardy, and M. Weigert. 1998. Regulation of anti-DNA B cells in recombination-activating gene-deficient mice. *J. Exp. Med.* 188:1247–1254.

24. Braun, U., K. Rajewsky, and R. Pelanda. 2000. Different sensitivity to receptor editing of B cells from mice hemizygous or homozygous for targeted Ig transgenes. *Proc. Natl. Acad. Sci. USA.* 97:7429–7434.

25. Li, H., Y. Jiang, E.L. Prak, M. Radic, and M. Weigert. 2001. Editors and editing of anti-DNA receptors. *Immunity.* 15:947–957.

26. Li, Y., H. Li, and M. Weigert. 2002. Autoreactive B cells in the marginal zone that express dual receptors. *J. Exp. Med.* 195:181–188.

27. Li, Y., H. Li, D. Ni, and M. Weigert. 2002. Anti-DNA B cells in MRL/lpr mice show altered differentiation and editing pattern. *J. Exp. Med.* 196:1543–1552.

28. Li, H., Y. Jiang, H. Cao, M. Radic, E.L. Prak, and M. Weigert. 2003. Regulation of anti-phosphatidylserine antibodies. *Immunity.* 18:185–192.

29. Shlomchik, M.J., J.A. Aucoin, D.S. Pisetsky, and M.G. Weigert. 1987. Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. *Proc. Natl. Acad. Sci. USA.* 84:9150–9154.

30. Hertz, M., and D. Nemazee. 1997. BCR ligation induces receptor editing in IgM+ IgD− bone marrow B cells in vitro. *Immunity.* 6:429–436.

31. Meffre, E., A. Schaefer, H. Wardemann, P. Wilson, E. Davis, and M.C. Nussenzweig. 2004. Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. *J. Exp. Med.* 199:145–150.

32. Foster, S.J., H.P. Brezinschek, R.I. Brezinschek, and P.E. Lipsky. 1997. Molecular mechanisms and selective influences that shape the kappa gene repertoire of IgM+B cells. *J. Clin. Invest.* 99:1614–1627.

33. Famer, N.L., T. Dorner, and P.E. Lipsky. 1999. Molecular mechanisms and selection influence the generation of the human V lambda J lambda repertoire. *J. Immunol.* 162:2137–2145.

34. Lang, J., M. Jackson, L. Teyton, A. Brunmark, K. Kane, and D. Nemazee. 1996. B cells are exquisitely sensitive to central tolerance and receptor editing induced by ultralow affinity, membrane-bound antigen. *J. Exp. Med.* 184:1685–1697.

35. Spanopoulou, E., C.A. Roman, I.M. Corcoran, M.S. Schlissel, D.P. Silver, D. Nemazee, M.C. Nussenzweig, S.A. Shinton, R.R. Hardy, and D. Baltimore. 1994. Functional immunoglobulin transgenes guide ordered B-cell differentiation in Rag-1-deficient mice. *Genes Dev.* 8:1030–1042.

36. Shlomchik, M., M. Maselli, H. Shan, M.Z. Radic, D. Pisetsky, A. Marshak-Rothstein, and M. Weigert. 1990. Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. *J. Exp. Med.* 171:265–292.

37. Ichiyoshi, Y., and P. Casali. 1995. Analysis of the structural correlates for self-antigen binding by natural and disease-related autoantibodies. In vitro expression of recombinant and/or murine-humanized human IgG. *Ann. NY Acad. Sci.* 764:328–341.

38. Shiokawa, S., F. Mortari, J.O. Lima, C. Nunez, F.E. Bertrand III, P.M. Kirkham, S. Zhu, A.P. Dasanayake, and H.W. Schroeder, Jr. 1999. IgM heavy chain complementarity-determining region 3 diversity is constrained by genetic and somatic mechanisms until two months after birth. *J. Immunol.* 162:6060–6070.

39. Zemlin, M., M. Klinger, J. Link, C. Zemlin, K. Bauer, J.A. Engler, H.W. Schroeder, Jr., and P.M. Kirkham. 2003. Expressed murine and human CDR-H3 intervals of equal length exhibit distinct repertoires that differ in their amino acid composition and predicted range of structures. *J. Mol. Biol.* 334:733–749.

40. Zhang, Z., M. Zemlin, Y.H. Wang, D. Munfus, L.E. Huye, H.W. Findley, S.L. Bridges, D.B. Roth, P.D. Burrows, and M.D. Cooper. 2003. Contribution of Vh gene replacement to the primary B cell repertoire. *Immunity.* 19:21–31.

41. Martin, F., and J.F. Kearney. 2000. B-cell subsets and the mature preimmune repertoire. Marginal zone and B1 B cells as part of a “natural immune memory.” *Immunol. Rev.* 175:70–79.

42. Nemazee, D. 2000. Receptor selection in B and T lymphocytes. *Annu. Rev. Immunol.* 18:19–51.

43. Williams, S.C., J.P. Frippiat, I.M. Tomlinson, O. Ignatovich, W. Schroeder, Jr., L.M. Corcoran, and M.C. Nussenzweig. 2004. Surrogate light chain expression of recombinant and/or murine-humanized human IgG. *Ann. NY Acad. Sci.* 1031:220–242.

44. Hieter, P.A., S.J. Korsmeyer, T.A. Waldmann, and P. Leder. 1984. Annu. Rev. Immunol. 18:19–51.

45. Williams, S.C., J.P. Frippiat, I.M. Tomlinson, O. Ignatovich, W. Schroeder, Jr., L.M. Corcoran, and M.C. Nussenzweig. 2004. Surrogate light chain expression of recombinant and/or murine-humanized human IgG. *Ann. NY Acad. Sci.* 1031:220–242.

46. Hieter, P.A., S.J. Korsmeyer, T.A. Waldmann, and P. Leder. 1984. Annu. Rev. Immunol. 18:19–51.