The antibody-combining site is formed by the interaction of the variable regions of the H and L chains; hence, replacement of either V changes the specificity of an antibody (Gay et al., 1993; Radic et al., 1993a; Tiegs et al., 1993). A characteristic feature of anti-DNA antibodies is that DNA contact is mediated primarily through the positively charged amino acid residue Arg in H-chain complementarity determining regions (CDRs; Jang et al., 1998; Radic et al., 1999, 1993b; Shlomchik et al., 1990). Hence, these Arg-containing H chains bind DNA regardless of most L chains (Ibrahim et al., 1995). However, several L chains in mouse can modify or veto anti-DNA binding when expressed with anti-DNA H chains. The human H chains expressed with these L chains also have relatively high arginine (Arg) content in the H chain complementarity determining region (H3), suggesting that receptor editing plays a role in establishing tolerance to DNA in humans.

Receptor editing is a mechanism of self-tolerance used in newly generated B cells. The expressed heavy (H) or light (L) chain of an autoreactive receptor is replaced by upstream V genes which eliminate or modify autoreactivity. Editing of anti-DNA receptors has been characterized in anti-DNA transgenic mouse models including 3H9, 3H9/56R, and their revertant 3H9GL. Certain L chains, termed editors, rescue anti-DNA B cells by neutralizing or modifying DNA binding of the H chain. This editing mechanism acts on the natural H chain repertoire; endogenous H chains with anti-DNA features are expressed primarily in combination with editor L chains. We ask whether a similar set of L chains exists in the human repertoire, and if so, do they edit H chains with anti-DNA signatures? We compared the protein sequences of mouse editors to all human L chains and found several human L chains similar to mouse editors. These L chains diminish or veto anti-DNA binding when expressed with anti-DNA H chains. The human H chains expressed with these L chains also have relatively high arginine (Arg) content in the H chain complementarity determining region (H3), suggesting that receptor editing plays a role in establishing tolerance to DNA in humans.
**Table 1.** Protein sequences of mouse editor, human potential editor and human non-editor light chains in this study

| Name               | FW1 | CDR1 | FW2 | CDR2 | FW3 | CDR3 |
|--------------------|-----|------|-----|------|-----|------|
| Mouse editor light chains |
| Vkb20              | STTVTQSPSLSMAGKVTRIC | ITSTDID------DN | WYQQKPGKPKLLIS | EGNTLRP------ | QVPFRSSGSGTDFTFVITENLSEDVAYYCL | QSDNL----- |
| Vbw20              | ETVTVQSPSLSAVGKVTIRC | ITSTDID------DN | WYQQKPGKPKLLIS | EGNTLRP------ | QVPFRSSGSGTDFTFVITENLSEDVAYYCL | QSDNL----- |
| Vkb1-4             | DTVVTQPSLSLAVQGRATISC | KAGSS-----------QGGTMH | WYQQKPGKPKLLIS | AASNLQS------ | GIPFRSGSGTDFTLENEVADDYTCQ | QSGNM----- |
| Vkb12-46           | DTVTVQPSLSVLQGVRVTITC | RASENYS------ALAA | WYQQKPGKPKLLIS | AATNLAD------ | GIPFRSGSGTDFTLENEVADDYTCQ | QSGNM----- |
| Vkb38c             | QVVTQPSLSLSAGKVRVTIC | QASOIN------YIA | WYQQKPGKPKLLIS | YTTSLQP------ | GIPFRSGSGTDFTLENEVADDYTCQ | QSDNL----- |
| VAX                | QVLVTQSS-SASFSGASKLTC | TLSSQIHT------YITE | WYQQKPGKPKLYME | LKKDQVCC-STD | GIPDRFSSGSGADYRLKISNIIQPEDEAYIC | GQGTIKQFV |

**Human potential editor light chains**

| Name               | FW1 | CDR1 | FW2 | CDR2 | FW3 | CDR3 |
|--------------------|-----|------|-----|------|-----|------|
| Vkb08/O18          | DTVTVQPSLSASVGRVTITC | QASDOIS------YLNI | WYQQKPGKPKLLIS | DSNLET------ | GIPFRSGSGTDFTLENEVADDYTCQ | QYQDL----- |
| Vkb11              | AITQTVQSPLSLAVGRVTITC | RASCGIN------DG | WYQQKPGKPKLLIS | AASNLQS------ | GIPFRSGSGTDFTLENEVADDYTCQ | QSYSTP----- |
| Vkb2/O12           | DTVTVQPSLSLSVKGRVTITC | QASQISS------SL | WYQQKPGKPKLLIS | AASNLQS------ | GIPFRSGSGTDFTLENEVADDYTCQ | QSYSTP----- |
| Vkb5-1             | LTVQIQLPFSAALSGKVLTC | LSSEQSHT------YITE | WYQQKPGKPKLYMK | VSDGSH-SKGD | GIPFRFNGSSGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Vkb5-2             | QVVTQPSLSLSASVGTLTC | LSSGQSN------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPDRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Vkb5-4             | QVVTQPSLSLSASSGKVLTC | LSSGQSS------YIA | WYQQKPGKPKLYMA | LNSGSH-SKGD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | ETWDN---- |
| Vkb5-6             | QVVTQPSLSLSASSGKVLTC | LSSGQSS------YIA | WYQQKPGKPKLYMA | LNSGSH-SKGD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | ETWDN---- |

**Human non-editor light chains**

| Name               | FW1 | CDR1 | FW2 | CDR2 | FW3 | CDR3 |
|--------------------|-----|------|-----|------|-----|------|
| Va2                | DTIVTQPPSSLTVPGQPASC | KSSQSLHSDG--QKTLYL | WYQQKPGKPKLLIS | EVSNRFS------ | GIPDRFSSGSGTDFTKISVREADEVVYCG | QSDNL----- |
| Va17               | DTVTQPPSSLTVPGQPASC | KSSQSLHSDG--QKTLYL | WYQQKPGKPKLLIS | EVSNRFS------ | GIPDRFSSGSGTDFTKISVREADEVVYCG | QSDNL----- |
| Va19               | DIVTVQPSLSLAVGRVTITC | KSSQSLHNA------------ | WYQQKPGKPKLLIS | LSNRNAS------ | GIPDRFSSGSGTDFTKISVREADEVVYCG | MQALTP----- |
| Va20               | DTVTVQPSLSLAVGRVTITC | RASQIGSN------YLA | WYQQKPGKPKVPLLL | AASTLQS------ | GIPDRFSSGSGTDFTKISVREADEVVYCG | QSYSTP----- |
| Va23               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Va26               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Va27               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Va30               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Va31               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Va32               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Va33               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Va34               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Va35               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |
| Va36               | DTVTVQPSLSLAVGRVTITC | RASQIVSN-------YKVD | WYQQKPGKPKLYMI | VSTGTVICSGKD | GIPFRFSSGSGGADYRLFSLQIDAEYHC | GSHTIDQVQ |

**RESULTS**

**Human anti-DNA L chain editors**

Human light chains similar to mouse editors were identified using the Ig Blast database (Table 1). Most, but not all, of these human L chains have Asps in CDRs (Table 1) and a low pI (Fig. 1). The location of Asps is the same in some of the mouse and human editor L chains, such as mouse Vkb38c and VAX and their respective human homologues Vkb08/O18 and VA5-1 (Table 1). Our results are consistent with the observation that Asps in L chain CDR regions are particularly important for editing of anti-DNA H chains (Radic et al., 1993b; Jang et al., 1996). The importance of CDR Asps for editing was originally shown in studies of the mouse chain editors (Gay et al., 1993; Radic et al., 1993a; Li et al., 2001). Substitution of any of the L chain CDR1 Asps by alanine in the L chain of an anti-DNA antibody resulted in increased sequence characteristics of editors—namely, the number and location of Asps in L-chain CDRs. Using this criterion, we identified several potential human editor L chains. We tested the ability of the human editors to modify DNA binding by H chains derived from monoclonal anti-DNA antibodies and found that they can silence anti-DNA H chains from both humans and mice. Because the human H3s are generated by mechanisms similar to those in mice, we reasoned that the human endogenous H-chain repertoire might also include H3-Arg. If editing of anti-DNAAs is a tolerance mechanism which operates in humans, then we would expect that these Arg-containing H chains are preferentially expressed with shown human editor light chains. This is indeed the case. This finding provides an understanding of how tolerance to DNA is maintained and, importantly, how the B cell repertoire is shaped in humans.
10- and 100-fold in 3H9 forward mutants 3H9/56R and 3H9/56R/76R (Radic et al., 1993b; Seal et al., 2000; Li et al., 2001; Chen et al., 2006). These anti-DNA H chains were expressed in mature B cells only when associated with editor L chains (Li et al., 2001, 2004).

To test whether the putative human editor L chains can edit 3H9 and its derivative H chain 3H9/56R, we examined the anti–double stranded DNA (dsDNA) and ANA activities of hybrid antibodies, where these H chains were expressed with each of four putative human editor L chains: V\( \alpha \)O8/O18, V\( \alpha \)O2/O12, V\( \alpha \)L11, and V\( \beta \)5-1 (Fig. 2). All four L chains vetoed the 3H9 H chain binding to dsDNA, and two L chains, V\( \alpha \)O2/O12 and V\( \beta \)5-1, decreased the affinity of 3H9/56R for DNA (Fig. 2).

Given the resemblance between the immune systems of man and mice, editors may be important in humans. Some human H chains have Args in their CDRs; hence, these H chains may have anti-DNA properties. We expressed four apparent anti-DNA human H chains with H3 Args and two mouse anti-DNA H chains 3H9 and 3H9/56R (Table 2) in combination with L chains that lack editor properties (i.e., V\( \alpha \)A27, V\( \alpha \)B3, and V\( \beta \)5-6) in 293 HEK cells. 11 out of 18 hybrid antibodies exhibited dsDNA or ANA activity (Fig. 2, B and D). We also cotransfected the same human H chains with the putative human editor L chains and examined the anti-dsDNA and ANA activities of the recombinant antibodies (Fig. 2). The DNA and ANA activities of human H chains

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**Table 2.** Expressed mouse and human H chain names and CDRs protein sequences

| Name     | VH/DH/JH         | CDR1     | CDR2                             | CDR3                             |
|----------|------------------|----------|----------------------------------|----------------------------------|
| Mouse anti-DNA H chains |                  |          |                                  |                                  |
| 3H9      | VMU-3.2/DSP2.13/JH3 | SSwMN    | RIYPRDGDIRNYNGKFDK               | ARSKYSVYMDY                      |
| 56R      | VMU-3.2/DSP2.13/JH3 | SSwMN    | RIYPRDGDIRNGKFDK                 | ARSKYSVYMDY                      |
| Human H chains |                  |          |                                  |                                  |
| OK57     | IGHV3-30-3/IGHD5-5/IGHJ4 | SYAHM    | VISYDGSKYYADSVKG                 | SKLRRTGCALCGY                    |
| OK13     | IGHV4-34/IGHD5/IGHJ3 | GYWS     | EINHSGS-TNYPSLKS                 | RRARGYSGYDRLANDAFDI              |
| KS60     | IGHV3-21/IGHD3-22/IGHJ3 | SYSMN    | SSSSSSYIYYADSVKG                 | AFDYRDRVRRGLADFADI               |
| OK7      | IGHV3-21/IGHD3-22/IGHJ1 | SYSMN    | SSSSSSYIYYADSVKG                 | RADYDSSGYHEYFQH                 |
L chain editing in human B cells | Kalinina et al.

H chain expressed with V\(\lambda\)5-6 exhibited a homogeneous nucleolar pattern, but 3H9/56R with V\(\lambda\)B3 showed a nucleolar pattern (Fig. 2 D). A specificity shift was previously observed with mouse antibodies; 3H9/56R H chain combined with most L chains bound dsDNA, but the 3H9/56R/V\(\lambda\)38c antibody bound Sm/ribonucleoprotein (RNP; Kishi et al., 2012).

B cells expressing Vk editor L chains have a biased H chain repertoire

The in vitro expression experiments described above demonstrate that human editor L chains can veto anti-DNA binding of H chains. Therefore, we asked whether these L chains edit endogenous anti-DNA H chains in the human B cell repertoire. In a previous study, we showed that the mouse editor

OK57 and OK13 were silenced by V\(\kappa\)O2/O12, V\(\kappa\)O8/O18, and V\(\kappa\)5-1 L chains, as were activities of H chain KS60 by V\(\kappa\)O8/O18 and V\(\kappa\)5-1L chains. H chain OK7, with one H3 Arg, exhibited neither DNA binding nor ANA activity when expressed with editor or non-editor L chains (not depicted), indicating that H3 Args are not always sufficient for DNA binding.

L chains can modify the avidity and the specificity of anti-DNA antibodies

We tested the ANA activity of the expressed antibodies described above. ANA activity correlated with DNA binding for 32 out of 35 expressed antibodies in our survey (Fig. 2). The ANA staining pattern varied with L chain usage; 3H9/56R H chain expressed with VA5-6 exhibited a homogeneous nuclear pattern, but 3H9/56R with V\(\kappa\)B3 showed a nucleolar pattern (Fig. 2 D). A specificity shift was previously observed with mouse antibodies; 3H9/56R. H chain combined with most L chains bound dsDNA, but the 3H9/56R/V\(\kappa\)38c antibody bound Sm/ribonucleoprotein (RNP; Kishi et al., 2012).
L chains are predominantly associated with endogenous H chains with H3 Args (putative anti-DNAs; Kalinina et al., 2011). We hypothesize that this bias is the result of receptor editing. Here, we performed this analysis in humans; we isolated 268 single peripheral blood B cells from healthy donors and sequenced the H and L chain genes expressed in each B cell. We then compared the H chains with the editor Vλ L chains VκO8/O18, VκO2/O12, and VκL11 to those with non-editor Vκ L chains (Table 1). The VH family distribution in B cells expressing the editor L chains was similar to that of B cells expressing non-editor L chains (Fig. 3). The VH3 family was expressed by >55% of B cells, followed by the VH4 family, which was expressed in >20%, and the VH1 family, which was expressed by 15%, and the expression of the VH5 and VH6 families was <5% of analyzed B cells. This distribution is the same as reported earlier (Brezinschek et al., 1997). Thus, the sorted population of peripheral B cells was representative of a human B cell repertoire. However, the B cells expressing editor L chains exhibited a significantly higher frequency of H chains with Args in H3 (60%) as compared with B cells expressing non-editor L chains (40%; P < 0.005, χ² test; Fig. 3 B).

Another feature of the edited antibodies of the mouse is a high frequency of distal Jκ genes (Radic et al., 1993a). We compared the frequency of different Jκ genes in editor L chains that were expressed in combination with H chains with H3 Args to that of the same L chains that were expressed with H chains without H3 Arg and found a higher frequency of distal Jκ usage in editor L chains associated with H chains which have H3 Args (Table 3; P = 0.075, χ² test). When we performed the same calculations for L chains that do not have editor properties, we did not find any change in Jκ usage between L chains that were expressed with H3 Arg H chains and H chains without H3 Arg (unpublished data).

**Lambda editors**

The mouse lambda locus includes an efficient editor, VAX, which has high sequence homology to the human Vλ5 lambda family. We isolated human Vλ5 cells using a mouse anti-VκX mAb (Fig. 4), and we sorted single B cells from the CD20+ VAX+ population (Fig. 4, right) and sequenced the L chain genes. Most of these B cells (80%) expressed L chains of the Vλ5 family. The expression of individual genes from the Vλ5 family in VλX+ sorted population was variable. Vλ5-1, the L chain in the Vλ5 family closest to VκX, is underrepresented in the human repertoire; we found only one productive rearrangement and no nonproductive ones in 226 Vλ5 cells. Given that the Vλ5 family comprises 1–2% of all B cells as identified by staining (Fig. 4), the frequency of Vλ5-1 expression is estimated to be 1 in 17,000 B cells. For Vλ5-2, we found only three productive and two nonproductive rearrangements.

**Table 3.** Jκ usage in L chain editors expressed with H chains which have H3 Args and H chains which lack Arg in H3

| Editor L chains | Jκ1 | Jκ2 | Jκ3 | Jκ4 | Jκ5 |
|----------------|-----|-----|-----|-----|-----|
| L11 (no H3 Args) | 2 (67%) | 1 (33%) | | | |
| L11 (H3 Args) | 2 (50%) | | | | |
| 02/012 (no H3 Args) | 2 (11%) | 10 (56%) | 1 (6%) | 3 (17%) | 2 (11%) |
| 02/012 (H3 Args) | 8 (31%) | 8 (31%) | 3 (12%) | 5 (19%) | 2 (8%) |
| 08/018 (no H3 Args) | 2 (25%) | 2 (25%) | 1 (13%) | 3 (38%) | |
| 08/018 (H3 Args) | 1 (8%) | 2 (15%) | | 6 (46%) | 4 (31%) |
| Total (no H3 Args) | 6 (21%) | 13 (43%) | 2 (7%) | 6 (21%) | 2 (7%) |
| Total (H3 Args) | 10 (23%) | 9 (21%) | 5 (12%) | 13 (30%) | 6 (14%) |

Figure 3. VH repertoire and percentage of VHs with H3 Args in sorted human B cells grouped by putative editor and non-editor L chain expression. (A) Frequency of each VH in B cells expressing potential editor (dark bars) and non-editor (light bars) Vκ L chains. (B) Percentage of H chains with H3 Args expressed with editor (dark bars) and non-editor (light bars) Vκ L chains. B cells were obtained from peripheral blood of five healthy human donors. H and L chain from the single-sorted Vκ-expressing B cells (CD20+Vκ+2) were amplified from cDNA and sequenced. n = 72 (100%) for B cells expressing editor Vκs and n = 194 (100%) for B cells expressing non-editor Vκs from five healthy donors. *, P = 0.01, X² test.
The Human L chain repertoire includes L chains that can modify DNA binding of H chain

A comparison of human and mouse L chain sequences identified human L chains that resemble the mouse editor L chains. To test whether these human L chains can function as editors to modify the DNA-binding capacity of a H chain, we expressed them in association with the mouse H chains known to bind DNA (Ibrahim et al., 1995). These human L chain editors reduce the affinity for DNA of mouse anti-DNA H chains. They also decrease the DNA binding of human H chains with H3 Args (Fig. 2). The degree to which DNA affinity is reduced ranges from no detectable DNA binding to intermediate levels of binding, depending on the particular L chain. The affinity range correlates with the number and location of Asps in the editor L chain; the most effective Asps are located in the CDRs, such as Asp$_{60}$ (L2) and Asp$_{96}$ (L3). These Asps are conserved between mouse and human editors (Table 1). The crystal structures of VAX antibodies (Li et al., 2007) show that Asp$_{96}$ interacts with H3 Arg. Conversely, the position of H-chain Args can also determine the efficiency of editing. Some Args, such as the Arg$_{96}$ in VH framework region 3, cannot be accessed by L chain; hence, antibodies that include a framework region Args in addition to CDR Args are only partially edited by any of the L chain editors.

**Table 4.** The frequency of human H chains with Arg in CDR3 in antibodies expressing Vk editor, Vk non-editor, and VA 5–6 human L chains from sorted peripheral blood

| B cell population that expresses | Number of H chain sequences analyzed | Number of H chains with Arg in CDR3 |
|----------------------------------|--------------------------------------|------------------------------------|
| Vk editors                       | 72                                   | 43 (60%)*                          |
| Vk non-editors                   | 194                                  | 77 (40%)                           |
| VA 5–6                           | 142                                  | 47 (33%)                           |

The number and percentage of H chains with Arg in CDR3 region in antibodies expressing Vk editor, Vk non-editor, and VA 5–6 L chains are shown. The CDR regions were identified according to the Kabat numbering scheme. *, P < 0.005, $\chi^2$ test.

L chain can modify the specificity of anti-DNA antibodies

Certain editor L chains veto DNA binding, thereby changing the specificity to an unknown antigen. Mouse editor Vk21D efficiently vetoes DNA binding and ANA activity of 3H9/56R H chain, but Vk38c paired with 3H9/56R acquires new auto-specificities, including binding to Sm/RNP and to the Golgi apparatus (Khan et al., 2011; Kishi et al., 2012). Another 3H9/56R/L chain combination, 3H9/56R/VAX, binds both DNA and MBP (Doyle et al., 2006). Here, we found that ANA staining patterns depend on human L chain usage and can shift from the nuclear homogeneous to the nucleolar pattern. Similarly, Wardemann et al. (2004) have shown that L chain exchange in certain antibodies changes the ANA patterns. Therefore, human L chains can modify both affinity and specificity of autoreactive antibodies.

Receptor editing in vivo

Receptor editing has been convincingly demonstrated in mice, and here we address whether receptor editing is effectively used to regulate anti-DNA B cells in humans. In mice, we showed that endogenous H chains that contain H3 Args are preferentially associated with editor L chains (Kalina et al., 2011). Here, we compared sequences of H chains expressed with putative editor human L chains to human L chains lacking editor properties and found that antibodies expressing editor human L chains have a higher frequency of H chains with H3 Args (60%) compared with antibodies expressing non-editor L chains (40%; P > 0.004, $\chi^2$ test; Fig. 3).
Because of the correlation between DNA binding and H3 Args, we infer that the observed difference in the H chain repertoire is a result of receptor editing in humans as well as in mice. Another indicator of receptor editing is a bias of JL usage to Jk3/Jk4/Jk5 of the κ L chain editors (Radic et al., 1993a) and we found that the editor L chains expressed with H chains with H3 Args have a trend toward a higher frequency of Jk3/Jk4/Jk5 rearrangements (Table 3).

Conservation of editors
The conservation of editor L chains between mouse and human is remarkable and argues for the importance of these L chains. VAX has little homology (<33% amino acid sequence identity) to either mouse λ or κV genes, suggesting that VAX, Vκ, and Vκ diverged at the same time (Sanchez et al., 1990). Nevertheless, VAX is conserved across species (Sanchez et al., 1990); mouse VAX and human Vκ-1 are 70% identical (Sanchez et al., 1991). Moreover, Vκ-1 and VAX share unusual characteristics such as a long L3 region and a stop codon just 3’ of the V coding region. The mouse anti-VAX mAbs cross-react with Vκ-1 expressing B cells, which allows us to calculate the frequency of the human Vκ (VAX-like) family expression in the mature B cell repertoire; this low frequency is also found for VAX in mice (Fig. 4). The Vκ-1 and the mouse VAX also share specificity. The mouse editor VAX alone and in combination with most H chains binds MBP (Galin et al., 1996a) and it was shown that a human IgM/Vκ antibody, that is recognized by an anti-VAX mAb, is also MBP-reactive (Noerager et al., 2001).

The putative human editor Vκ08/018 shares properties with the mouse editor Vκ38c both in sequence homology and editing properties. Moreover, studies of Ig L chain amyloidosis documented that Vκ08/018 L chains form amyloidogenic proteins (Connors et al., 2007). Mouse Vκ38c antibodies were shown to be secreted in the form of extracellular aggregates called spheroids that stain with the amyloid-selective dye thioflavin T (Khan et al., 2011). The similarities between mouse and human editors indicate that these editors may perform similar functions.

Conclusions
Our data demonstrate that the human L chain repertoire includes editor L chains as do mice and presumably other species. Our finding that human editor L chains associate with H chains which have an elevated frequency of H3 Args suggests that humans use receptor editing to regulate anti-DNA B cells. We also show here that receptor editing shapes the B cell repertoire. Editor L chains rescue expression of anti-DNA H chains, and likewise anti-DNA H chains rescue some editor L chains. Some antibodies that express editor L chains, such as mouse VH1783/VAX, are frequently used in broadly neutralizing antiviral antibodies (Lee et al., 2008). Another antibody with the same editor L chain MW1/VAX is reactive to poly-Q repeats (Li et al., 2007). Therefore, editing of self-reactive antibodies is important for both protective immunity and tolerance.

MATERIALS AND METHODS
Single B cell isolation and sorting. Samples of peripheral blood were collected from 5 healthy donors (22–50 yr old). The use of human blood was approved by the University of Chicago, Institutional Review Board (protocol 14801B), and informed consent was obtained from all participants. Mononuclear cells were isolated from peripheral blood using lymphocyte separation medium (Corning) according to the manufacturer’s instructions. Purified mononuclear cells were stained with anti-human antibodies FITC-labeled anti-Igκ (BD), PE-labeled anti-Igκ (BD), PE-Cy7-labeled CD20 (BD), and Alexa Fluor 647–conjugated anti-VAX monoclonal antibody (10C5), specific for the mouse VAX gene segment (provided by P-A. Cazenave; Sanchez et al., 1991). Single cell sorting was performed as described in Wardemann et al. (2003) with slight modifications. Single cells were sorted on a FACSARia II (BD) into 96-well PCR plates containing 4 µl lysin solution (0.5× PBS containing 10 mM dithiothreitol and 8.4 U RNase Inhibitor [New England Biolabs, Inc.]); plates were immediately frozen on dry ice and stored at −80°C. Single B cells expressing Vκ genes (CD20+Vκc) and VκX B cells (CD20+VAXc) were sorted separately.

cDNA synthesis, PCR, and sequencing. For cDNA synthesis, RNA from single cells was reverse transcribed at 37°C for 55 min with 150 ng random hexamer primer (Integrated DNA Technologies), 0.5 µl dNTP mix (10 mM each, Fermentas), 1 µl of 0.1 M dithiothreitol (Invitrogen), 0.5% (vol/vol) Nonidet P-40, 5.6 U RNase inhibitors (New England Biolabs, Inc.), and 50 µl Superscript III reverse transcription (Invitrogen) in a total volume of 14 µl. Heavy and light chain genes from single cells were amplified by PCR from cDNA using two rounds of reactions with previously published nested primers (Smith et al., 2009); 40 µl reactions included 10 pmol of each primer and 0.4 µl of JumpStart Taq DNA polymerase [Sigma–Aldrich]). Aliquots of second PCR products were purified by incubation with 10 U alkaline phosphatase, calf intestinal (CIP; New England Biolabs, Inc.), and 20 U exonuclease I (New England Biolabs, Inc.) in 1× NEB 3 buffer for 15 min at 37°C and sequenced using the reverse primers at the University of Chicago sequencing core: VH, DH, JH, VL, and JL usage was identified using igBLAST.

Generation of recombinant antibodies. The recombinant antibodies were generated as described previously (Smith et al., 2009). In brief, PCR products of particular VH, Vκ, and VA genes were cloned into IgG1, Igκ, or Igλ expression vectors, respectively. Several expressed Vκ (A27, L11, O8/018, and O2/012) and VA (5–6) genes were provided by the Patrick Wilson laboratory (University of Chicago, Chicago, IL). Equimolar amounts of expression vectors containing the desired IgH or Igκ genes were cotransfected into the 293 cell line using the polyethylenimine, linear, mol wt–25,000 (PEI) transfection reagent (Polysciences, Inc.). The recombinant antibodies were purified from the cell supernatant 5 d after transfection using Pierce Protein A Trisacryl Resin (Thermo Fisher Scientific) and concentrated with Amicon Ultra Centrifugal Filters (Millipore) according to the manufacturer’s instructions. Antibody concentrations were determined using a NanoDrop Spectrophotometer ND-1000 (NanoDrop Technologies).

ELISA for dsDNA binding. Antibody concentrations were adjusted to 10 µg/ml, and four consecutive 1:3 dilutions in blocking solution (1% BSA in PBS) were prepared. ELISA assays were performed as described previously (Radic et al., 1993a). Immulon 4 HBX ELISA plates (Thermo Fisher Scientific) were coated with 10 µg avidin D (Vector Laboratories) in PBS at 4°C overnight, blocked in PBS with 1% BSA at RT for 2 h, and washed with PBS with 0.05% Tween (PBS-T). Biotinylated dsDNA was bound to avidin-coated plates at 37°C for 1.5 h. Diluted antibodies were applied for 1.5 h at 37°C; plates were washed three times, and DNA–Ab complexes were detected with alkaline phosphatase–conjugated anti-human IgG (H+L) Ab (Bio-Rad Laboratories). After absorption for 90 min, plates were washed and the remaining anti-dsDNA Abs were quantified using AP substrate (Sigma–Aldrich). The OD was determined at 405 nm.

ANA assay. Antibody concentrations were adjusted to 50 µg/ml to test for antinuclear antigen binding using a Hep-2 ANA kit (Bion Enterprises, LTD) according to the manufacturer’s instructions. Slides were examined on a DMR fluorescence microscope (Leica). Images were captured using a 63×/1.40–0.60 oil objective (Leica) and a 10×/25 ocular (Leica), with a Retiga 200R camera (Q Imaging) and Q Capture Pro (Media Cybernetics) imaging software.
Statistical analysis. P-values for Ig gene repertoire analysis and arginine content in H3 were calculated using the χ² test.

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