Light chain editors of anti-DNA receptors in human B cells

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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.

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Abbreviations used: ANA, antinuclear antibody; CDR, complementarity determining region; dsDNA, double stranded DNA; MBP, myelin basic protein; pI, isoelectric point.

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., 1989, 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 DNA binding when paired with anti-DNA H chains (Li et al., 2001). These L chains are called editors and share functionally consequential structural characteristics such as a low isoelectric point (pI) and negatively charged aspartate residues (Asp) in CDRs (Li et al., 2001).

Editor L chains were originally discovered in anti-DNA H-chain transgenic models (Gay et al., 1993; Radic et al., 1993a). The majority of peripheral B cells bearing anti-DNA transgenic H chains were paired with editor L chains, indicating that only those B cells that edited their receptors were allowed to migrate to the periphery. Editing of anti-DNA receptors also influences the endogenous mouse repertoire; antibodies which use editor L chains have characteristics of lupus anti-DNA antibodies—namely, a high frequency of H chains with Args in H3 (H3-Arg) (Kalinina et al., 2011).

Here, we studied the human antibody repertoire to determine whether it contains L chains similar to the mouse editors, whether such human L chains silence or modify anti-DNA activity, and whether antibodies that express these L chains have a high frequency of H chains with H3 Arg relative to the total human antibody repertoire. We used mouse editor L chain sequences as a guideline for identifying human editor L chains. We focused on the prominent

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Table 1. Protein sequences of mouse editor, human potential editor and human non-editor light chains analyzed in this study

Name	FW1	CDR1	FW2	CDR2	FW3	CDR3
Mouse editor light chains						
VκBt20	STTVTQSPASLSMAIGEKVTIRC	ITSTDIDD------DMN	WYQQKPGEPPKLLIS	EGNTLRP-----	GVPSRFSSSGYGTDFVFTIENMLSEDVADYYCL	QSDNLP----
VκBw20	ETTVTQSPASLSVATGEKVTIRC	ITSTDIDD------DMN	WYQQKPGEPPKLLIS	EGNTLRP-----	GVPSRFSSSGYGTDFVFTIENTLSEDVADYYCL	QSDNMP----
Vκ21-4	DIVLTQSPASLAVSLGQRATISC	KASQS-VDYD-GDSYMN	WYQQKPGQPPKLLIY	AASNLES-----	GIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQ	QSNEDP----
Vκ12-46	DIQMTQSPASLSVSVGETVTITC	RASENIYS------NLA	WYQQKQGKSPQLLVY	AATNLAD-----	GVPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQ	HFWGTP----
VκGj38c	DIQMTQSPSSLSASLGGKVTITC	KASQDINK------YIA	WYQHKPGKGPRLLIH	YTSTLQP-----	GIPSRFSGSGSGRDYSFSISNLEPEDIATYYCL	QYDNLL----
VλX	QLVLTQSS-SASFSLGASAKLTC	TLSSQHST-----YTIE	WYQQQPLKPPKYVME	LKKDGSH-STGD	GIPDRFSGSSSGADRYLSISNIQPEDEAIYIC	GVGDTIKEQFV
Human potential editor light chains						
VκO8/O18	DIQMTQSPSSLSASVGDRVTITC	QASQDISN------YLN	WYQQKPGKAPKLLIY	DASNLET-----	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNLP----
VκL11	AIQMTQSPSSLSASVGDRVTITC	RASQGIRN------DLG	WYQQKPGKAPKLLIY	AASSLQS-----	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	LQDYNYP----
VκO2/O12	DIQMTQSPSSLSASVGDRVTITC	RASQSISS------YLN	WYQQKPGKAPKLLIY	AASSLQS-----	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTP----
Vλ5-1	LPVLTQPPSASALLGASIKLTCT	LSSEHST------YTIE	WYQQRPGRSPQYIMK	VKSDGSH-SKGD	GIPDRFMGSSSGADRYLTFSNLQSDDEAEYHC	GESHTIDGQVG
Vλ5-2	QPVLTQPPSASASLGASVTLTCT	LSSGYSN------YKVD	WYQQRPGKGPRFVMR	VGTGGIVGSKGD	GIPDRFSVLGSGLNRYLTIKNIQEEDESDYHC	GADHGSGSNFV
Vλ5-4	QPVLTQSSSASASLGSSVKLTCT	LSSGHSS------YIIA	WHQQQPGKAPRYLMK	LEGSGSY-NKGS	GVPDRFSGSSSGADRYLTISNLQFEDEADYYC	ETWDSNT----
Vλ5-6	QLVLTQSPSASASLGASVKLTCT	LSSGHSS------YAIA	WHQQQPEKGPRYLMK	LNSDGSH-SKGD	GIPDRFSGSSSGAERYLTISSLQSEDEADYYC	QTWGTG-----
Human non-editor light chains						
VκA2	DIVMTQTPLSLSVTPGQPASISC	KSSQSLLHSD-GKTYLY	WYLQKPGQPPQLLIY	EVSNRFS-----	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQSIQLP----
VκA17	DVVMTQSPLSLPVTLGQPASISC	RSSQSLVYSD-GNTYLN	WFQQRPGQSPRRLIY	KVSNRDS-----	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQGTHWP----
VκA19	DIVMTQSPLSLPVTPGEPASISC	RSSQSLLHSN-GYNYLD	WYLQKPGQSPQLLIY	LGSNRAS-----	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQALQTP----
VκA20	DIQMTQSPSSLSASVGDRVTITC	RASQGISN------YLA	WYQQKPGKVPKLLIY	AASTLQS-----	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC	QKYNSAP----
VκA23	DIVMTQTPLSSPVTLGQPASISC	RSSQSLVHSD-GNTYLS	WLQQRPGQPPRLLIY	KISNRFS-----	GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYC	MQATQFP----
VκA26	EIVLTQSPDFQSVTPKEKVTITC	RASQSIGSS------LH	WYQQKPDQSPKLLIK	YASQSFS-----	GVPSRFSGSGSGTDFTLTINSLEAEDAATYYC	HQSSSLP----
VκA27	EIVLTQSPGTLSLSPGERATLSC	RASQSVSSS-----YLA	WYQQKPGQAPRLLIY	GASSRAT-----	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSP----
VκA30	DIQMTQSPSSLSASVGDRVTITC	RASQGIRN------DLG	WYQQKPGKAPKRLIY	AASSLQS-----	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNSYP----
VκB3	DIVMTQSPDSLAVSLGERATINC	KSSQSVLYSSNNKNYLA	WYQQKPGQPPKLLIY	WASTRES-----	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	QQYYSTP----
VκL1	DIQMTQSPSSLSASVGDRVTITC	RASQGISN------YLA	WFQQKPGKAPKSLIY	AASSLQS-----	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQYNSYP----
VκL2	EIVMTQSPATLSVSPGERATLSC	RASQSVSSN------LA	WYQQKPGQAPRLLIY	GASTRAT-----	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	QQYNNWP----
VκL5	DIQMTQSPSSVSASVGDRVTITC	RASQGISS------WLA	WYQQKPGKAPKLLIY	AASSLQS-----	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFP----
VκL6	EIVLTQSPATLSLSPGERATLSC	RASQSVSS------YLA	WYQQKPGQAPRLLIY	DASNRAT-----	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	QQRSNWP----
VκL8	DIQLTQSPSFLSASVGDRVTITC	RASQGISS------YLA	WYQQKPGKAPKLLIY	AASTLQS-----	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	QQLNSYP----
VκL9	AIRMTQSPSSFSASTGDRVTITC	RASQGISS------YLA	WYQQKPGKAPKLLIY	AASTLQS-----	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	QQLNSYP----
VκL12a	DIQMTQSPSTLSASVGDRVTITC	RASQSISS------WLA	WYQQKPGKAPKLLIY	KASSLES-----	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC	QQYNSYSP---
VκL4/18a	AIQLTQSPSSLSASVGDRVTITC	RASQGISS------ALA	WYQQKPGKAPKLLIY	DASSLES-----	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQFNSYP----
L23	AIRMTQSPFSLSASVGDRVTITC	WASQGISS------YLA	WYQQKPAKAPKLFIY	YASSLQS-----	GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC	QQYYSTP----
L24	VIWMTQSPSLLSASTGDRVTISC	RMSQGISS------YLA	WYQQKPGKAPELLIY	AASTLQS-----	GVPSRFSGSGSGTDFTLTISCLQSEDFATYYC	QQYYSFP----

Framework (FW) and CDR regions are identified according to Kabat definition, Asp residues (D) are underlined in CDR regions. Four Ds in CDRs are not underlined: (1) in Vk21-4 CDR1, (2) in VkA19 CDR1, (3) inVkL6 CDR2, and (4) in VkL4/18a CDR2. Not all human L chains listed in the table, but only those that were found in our survey.

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-Args. If editing of anti-DNAs is a tolerance mechanism which operates in humans, then we would expect that these Arg-containing H chains are preferentially expressed with 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.

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 Vκ38c and VλX and their respective human homologues VκO8/O18 and Vλ5-1 (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 L chain editors (Gay et al., 1993; Radic et al., 1993a; Li et al., 2001). Substitution of any of the L chain CDR1 (L1) Asp by alanine in the L chain of an anti-DNA antibody resulted in increased

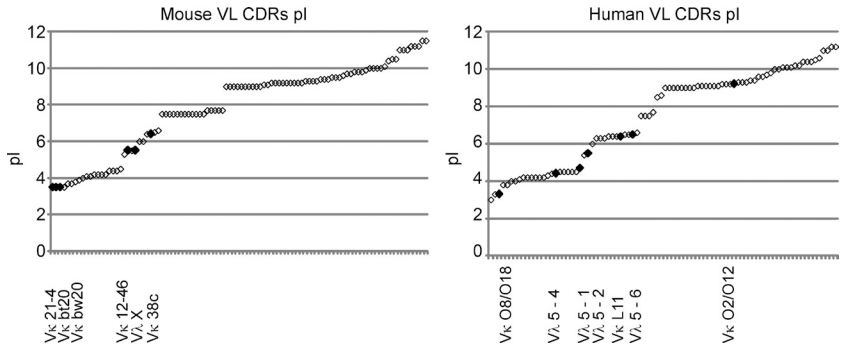

Figure 1. The pI of CDR regions of mouse and human L chains. Mouse and human L chains are plotted in order of increasing CDR pI. CDRs are identified according to the Kabat definition. Mouse editor and human putative editor L chain CDR pI values are shown in solid blocks and these L chains are labeled on the X axis. The pI values for the remaining L chains are shown as open data points to place the editors in context within the full set of L chains in mouse and in human.

DNA binding (Jang et al., 1998). The ability of Asp in L2 to interact with Arg in a H3 was revealed in the crystal structure of an anti–poly-Q antibody (Li et al., 2007).

Asp in L chain CDRs is neither necessary nor sufficient for editing; we found human L chain editors without Asps that can edit, demonstrating that editing is not restricted to the presence of Asps. For example, the human L chain, VκO2/O12, homologous to mouse editor L-chain Vκ12-46, does not have CDR Asps but does veto DNA-binding and antinuclear antibody (ANA) activity of anti-DNA H chains (Table 1 and Fig. 2). Conversely, a homologue of the mouse editor VλX, human Vλ5-6, has two Asps in L2 but does not veto DNA or ANA activity of either mouse or human H chains with H3-Args (Table 1 and Fig. 2). Human Vλ5-6 and mouse VλX have a similar VL sequence, including the two Asps in L2 (also found in Vλ5-1), but are dissimilar in L3. Both VλX and its closest human homologue Vλ5-1 have long L3s that contain Asp in site 96, whereas Vλ5-6 has a short L3 and no L3 Asp. As shown for VλX junctional variants, L3 is of paramount importance for editing (Radic et al., 2013).

L chains veto and modify DNA binding

The affinity of an antibody H chain for DNA depends mainly on the number and location of Args, as has been shown by mutagenesis; substitution of the 3H9 H-chain Arg with the germline-encoded VH amino acid glutamine abolishes DNA-binding, whereas addition of Args enhances DNA binding by 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: VκO8/O18, VκO2/O12, VκL11, and Vλ5-1 (Fig. 2). All four L chains vetoed the 3H9 H chain binding to dsDNA, and two L chains, VκO2/O12 and Vλ5-1, 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., VκA27, VκB3, and Vλ5-6) 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

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	RIYPRDGDINYNGKFKD	ARSKYSYVMDY
56R	VMU-3.2/DSP2.13/JH3	SSWMN	RIYPRDGRINYNGKFKD	ARSKYSYVMDY
Human H chains				
OK57	IGHV3-30-3/IGHD5-5(18)/IGHJ4	SYAMH	VISYDGSNKYYADSVKG	SKLRRTGALCGY
OK13	IGHV4-34IGHD/D5-5/IGHJH3	GYYWS	EINHSGS-TNYNPSLKS	RRARGYSYGDRLANDAFDI
KS60	IGHV3-21/IGHD3-22/IGHJ3	SYSMN	SISSSSSYIYYADSVKG	AFDYDRRVRRGLDAFDI
OK7	IGHV3-21/IGHD3-22/IGHJ1	SYSMN	SISSSSSYIYYADSVKG	RADYYDSSGYHEYFQH

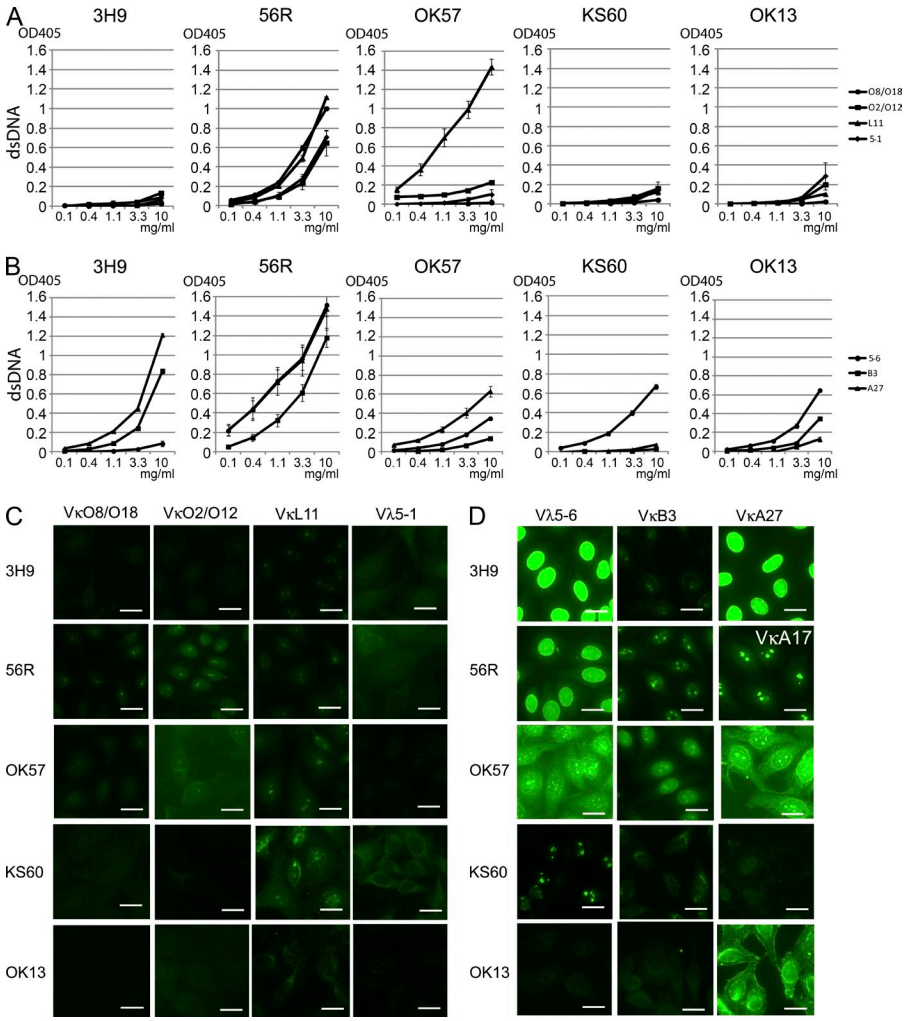

Figure 2. dsDNA binding and ANA staining of the human/mouse hybrid antibodies. (A and C) dsDNA binding curves (A) and ANA staining (C) for the four putative human L chain editors (Vκ018, Vκ012, VκL11, and Vλ5-1) as expressed with two mouse anti-DNA H chains (3H9 and 3H9/56R) and three human H chains (OK57, KS60, and OK13; Table 2). (B and D) dsDNA binding curves (B) and ANA staining (D) for three human L chains (Vλ5-6, VκB3, and VκA27, and for some experiments VκA17) which lack an editor signature, as expressed with the H chains used in testing the putative editor L chains. The hybrid antibodies were tested for reactivity to dsDNA by ELISA. The initial antibody concentration was 10 µg/ml, with four consecutive 1:3 dilutions for ELISA (A and B). Means of at least three independent experiments are plotted, with error bars representing the standard error. For testing of ANAs by HEp2 fluorescence (ANA, C and D), antibodies were used at 50 µg/ml. Bars, 2.0 µm. The ANA staining patterns were defined according to Bradwell and Hughes (2007; C and D). Data are representative of three to five independent experiments.

OK57 and OK13 were silenced by VκO2/O12, VκO8/O18, and Vλ5-1 L chains, as were activities of H chain KS60 by VκO8/O18 and Vλ5-1L 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 Vλ5-6 exhibited a homogeneous nuclear pattern, but 3H9/56R with VκB3 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/Vκ38c antibody bound Sm/ribonucleoprotein (RNP; Kishi et al., 2012).

B cells expressing Vκ 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

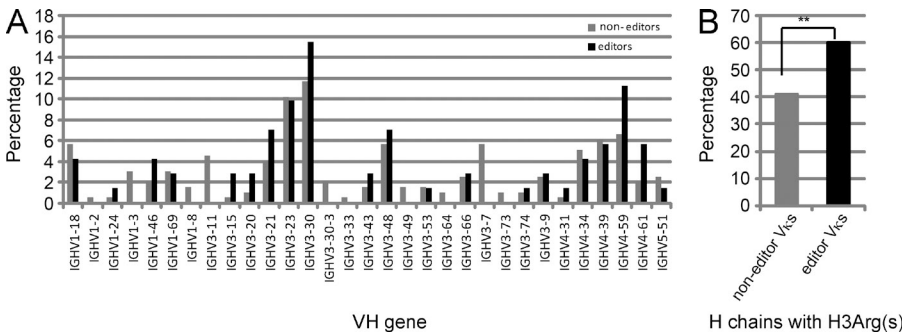

Figure 3. VH repertoire and percentage of VHs with H3 Args in sorted human B cells grouped by putative editor and non-editor L chain expression. (A) Frequency of each VH in B cells expressing potential editor (dark bars) and non-editor (light bars) Vκ L chains. (B) Percentage of H chains with H3 Args 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 ($CD20^+V\kappa^+$) 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, χ^2 test.

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 Vκ 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%; $P < 0.005$, χ^2 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; $P = 0.075$, χ^2 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, VλX, which has high sequence homology to the human Vλ5 lambda family. We isolated human Vλ5 cells using a mouse anti-VλX mAb (Fig. 4), and we sorted single B cells from the $CD20^+$ $V\lambda X^+$ 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 $V\lambda X^+$ sorted population was variable. Vλ5-1, the L chain in the Vλ5 family closest to VλX, 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%)			2 (50%)	
O2/O12 (no H3 Args)	2 (11%)	10 (56%)	1 (6%)	3 (17%)	2 (11%)
O2/O12 (H3 Args)	8 (31%)	8 (31%)	3 (12%)	5 (19%)	2 (8%)
O8/O18 (no H3 Args)	2 (25%)	2 (25%)	1 (13%)	3 (38%)	
O8/O18 (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%)

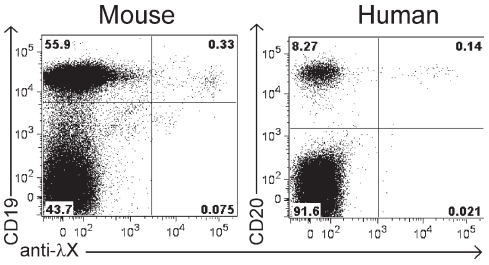

Figure 4. VλX⁺ B cells in mouse and human. Splenocytes from B6 WT (left) mice were stained with Phycoerythrin/Cy7 anti-CD19 and Alexa Fluor 647 anti-λX monoclonal antibodies (Sanchez et al., 1991). Human peripheral blood B cells (right) were stained with Phycoerythrin/Cy7 anti-CD20 and Alexa Fluor 647 anti-λX monoclonal antibodies (Sanchez et al., 1991).

The frequencies of Vλ5-4 and Vλ5-6 genes are 1 in 800 and 1 in 85 B cells, respectively. These data are in agreement with the analysis of VL gene expression in a large number of healthy individuals using a L-chain microarray chip, on which neither Vλ5-1 nor Vλ5-2 were detected (Schoettler et al., 2012). Vλ5-1 L chain was detected on the L-chain microarray chip analysis of B cells from SLE patients (Schoettler et al., 2012). Low Vλ5-1 and Vλ5-2 expression could be explained by the stop codon at the 3′ end of these V genes. This restricts the possibility of an in-frame rearrangement to just one register. Also, Vλ5-1 is most proximal to the JC genes in the lambda locus (Kawasaki et al., 1997); therefore, this V gene will be deleted by most lambda rearrangements. Alternatively, Vλ5-1 might be edited because it binds proteins such as myelin basic protein (MBP: Galin et al., 1996a,b). A majority (202 out of 226) of the B cells from the CD20⁺VλX⁺ population expressed the Vλ5-6 L chain. This L chain has high homology to the mouse editor VλX, except for the H3 region, but as we showed by in vitro expression experiments, Vλ5-6 does not silence anti-DNA or ANA activity when paired with mouse anti-DNA H chains or with human H chains with Args in H3 (Fig. 2, B and D). A comparative analysis of frequencies of H chains with H3 Args in antibodies that express Vλ5-6 to that of antibodies expressing Vκ editor L chains also indicates that Vλ5-6 belongs to the non-editor group. In antibodies expressing Vλ5-6, the percentage of H chains with H3 Args was lower (33%) than that of antibodies expressing Vκ editor L chains (60%; $P > 0.005$, Chi-Square test; Table 4).

Table 4. The frequency of human H chains with Arg in CDR3 in antibodies expressing Vκ editor, Vκ non-editor, and Vλ 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
Vκ editors	72	43 (60%)*
Vκ non-editors	194	77 (40%)
Vλ 5-6	142	47 (33%)

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

DISCUSSION

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 an 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 VλX antibodies (Li et al., 2007) show that Asp_{60} interacts with H3 Arg. Conversely, the position of H-chain Args can also determine the efficiency of editing. Some Args, such as the Arg_{76} 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.

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 Vκ21D efficiently vetoes DNA binding and ANA activity of 3H9/56R H chain, but Vκ38c 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/VλX, 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 which contain H3 Args are preferentially associated with editor L chains (Kalinina 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%; $P > 0.004$, χ^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 Jκ3/Jκ4/Jκ5 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 Jκ3/Jκ4/Jκ5 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. VλX has little homology (<33% amino acid sequence identity) to either mouse λ or κ V genes, suggesting that VλX, Vλ, and Vκ diverged at the same time (Sanchez et al., 1990). Nevertheless, VλX is conserved across species (Sanchez et al., 1990); mouse VλX and human Vλ5-1 are 70% identical (Sanchez et al., 1991). Moreover, Vλ5-1 and VλX share unusual characteristics such as a long L3 region and a stop codon just 3′ of the V coding region. The mouse anti-VλX mAbs cross-react with Vλ5-expressing B cells, which allows us to calculate the frequency of the human Vλ5 (VλX-like) family expression in the mature B cell repertoire; this low frequency is also found for VλX in mice (Fig. 4). The Vλ5-1 and the mouse VλX also share specificity. The mouse editor VλX alone and in combination with most H chains binds MBP (Galin et al., 1996a) and it was shown that a human IgM/Vλ antibody, that is recognized by an anti-VλX mAb, is also MBP-reactive (Noerager et al., 2001).

The putative human editor VκO8/O18 shares properties with the mouse editor Vκ38c both in sequence homology and editing properties. Moreover, studies of Ig L chain amyloidosis documented that VκO8/O18 L chains form amyloidogenic proteins (Connors et al., 2007). Mouse Vκ38c antibodies were shown to be secreted in the form of extracellular aggregates called spherons 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 VH7183/VλX, are frequently used in broadly neutralizing antiviral antibodies (Lee et al., 2008). Another antibody with the same editor L chain MW1/VλX 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-Igλ (BD), PE-Cy7–labeled CD20 (BD), and Alexa Fluor 647–conjugated anti-VλX monoclonal antibody (10C5), specific for the mouse VλX 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 lysis 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 ($CD20^+$ $V\kappa^+$) and $V\lambda X^+$ B cells ($CD20^+$ $V\lambda X^+$) 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 U 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 Vλ genes were cloned into IgG1, Igκ, or Igλ expression vectors, respectively. Several expressed Vκ (A27, L11, O8/O18, and O2/O12) and Vλ (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 IgL 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 Spectraphotometer 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 χ^2 test.

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