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Artificial immunoglobulin light chain with potential to associate with a wide variety of immunoglobulin heavy chains

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ABSTRACT

Immunoglobulins play important roles in antigen recognition during the immune response, and the complementarity-determining region (CDR) 3 of the heavy chain is considered as the critical antigen-binding site. We previously developed a statistical protocol for the extensive analysis of heavy chain variable region repertoires and the dynamics of their immune response using next-generation sequencing (NGS). The properties of important antibody heavy chains predicted *in silico* by the protocol were examined by gene synthesis and antibody protein expression; however, the corresponding light chain that matches with the heavy chain could not be predicted by our protocol. To understand the dynamics of the heavy chain and the effect of light chain pairing on it, we firstly tried to obtain an artificial light chain that pairs with a broad range of heavy chains and then analyzed its effect on the antigen binding of heavy chains upon pairing. During the pre-B cell stage, the surrogate light chain (SLC) could pair with the nascent immunoglobulin μ heavy chains (Ig-μH) and promote them to function in the periphery. On the basis of this property, we designed several versions of genetically engineered "common light chain" prototypes by modifying the SLC structure. Among them, the mouse-derived VpreB1λ5Cκ light chain showed acceptable matching property with several different heavy chains without losing specificity of the original heavy chains, though the antigen affinities were variable. The extent of matching depended on the heavy chain; surprisingly, a specific heavy chain (IGHV9-3) could match with two different conventional Vκs (IGKV3-2*01 and IGKV10-96*01) without losing the antigen affinities, whereas another heavy chain (IGHV1-72) completely lost its antigen affinities by the same matching. Thus, the results suggested that the antigen recognition of the heavy chain is variably affected by the paired light chain, and that the artificial light chain, Mm_VpreB1λ5Cκ, has the potential to be a "common light chain", providing a novel system to analyze the effects of light chains in antigen recognition of heavy chains.

1. Introduction

The variable regions of antibody heavy chains (IgHs) show enormous diversity by V-D-J gene rearrangements that include random nucleotide additions in the complementarity-determining region (CDR) 3 [1,2]. The variable regions of antibody light chains (IgLs) are less diverse than IgHs because of the lack of D_L gene segments and poor nucleotide insertions [3].

The use of next-generation sequencing (NGS) to analyze antibody repertoires is advantageous for rapid and extensive identification of antigen-specific antibodies [4–7]. We previously reported a method for the holistic analysis of IgH and IgL repertoires respectively from mice and humans [8,9]. In contrast to the recent single cell-based NGS repertoire analyses [10,11], this method has an advantage of easy and inexpensive handling of huge numbers of IgH and IgL sequences, but has a disadvantage of inability to determine the pairing of IgH and IgL.

In addition, we developed an intelligible method for detecting

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antigen-responding IgH repertoires by analyzing dynamic changes in whole antibodies in individual mice during 4-hydroxy-3-nitrophenyl acetyl (NP) hapten and chicken γ-globulin (CGG) antigen immunization and found that the major responding IgHs were IGHV1-72/IGHD1-1/IGHJ2 for NP-hapten and IGHV9-3/IGHD3-1/IGHJ2 for CGG-carrier protein [8]. The former antigen-antibody interaction was extensively analyzed by the crystallography [12], whereas the latter interaction was a new finding. We confirmed the latter interaction by gene synthesis of NGS reads and antibody protein expression followed by ELISA test. During the course of the study, we realized the lack of information on the mode of interaction between IgH and IgL, and the effect of IgL pairing on the antigen binding affinity of IgH.

In this report, we describe examples of IgH/IgL matching in terms of the antigen-binding of IgHs specific to NP-hapten and CGG-carrier antigens. In addition, we attempted the construction of "common light chain" models that preserve the potential to associate with a wide variety of IgHs.

During the early stage of B cell development, the V-D-J rearranged μH pairs with the surrogate light chains (SLCs), VpreB and λ5, to form the pre-B cell receptor (pre-BCR) [13,14]. Pre-BCR serves as an important checkpoint and participates in IgH repertoire selection, which is crucial for B cell maturation and migration to the periphery [15–17]. These findings suggest that the SLC can bind to various IgHs and select them to function in the peripheral immune system [18]. Based on this, we utilized SLC molecules to construct "common light chain" models, and examined their matching ability to various IgHs and their effect on antigen binding affinities of IgHs in comparison to those of conventional IgL. These results would provide useful information on antigen recognition of IgH/IgL pairs and on the optimization of antibody drugs.

2. Materials and Methods

2.1. Construction of VpreBλ5Cκ light chains

On the basis of the SLC structures, the engineered VpreBλ5Cκ light chains were constructed by the following processes: 1. the "tail" domain of λ5 was cut; 2. the redundant non-Ig part of VpreB was cut and removed; 3. the terminal of VpreB and the "tail" of λ5 were spliced to form a new CDR-L3 loop; 4. the Arg101Tyr mutation was introduced into VpreB; 5. the engineered VpreB-λ5 light chain variable region (VpreBλ5) was connected to the human Igκ constant region (Cκ) already present in the antibody expression vector (Mammalian PowerExpress System, TOYOBO MPH-101).

2.2. DNA plasmids and antibody gene synthesis

Antibody gene synthesis was performed as described previously [8]. For details, see Supplementary Methods.

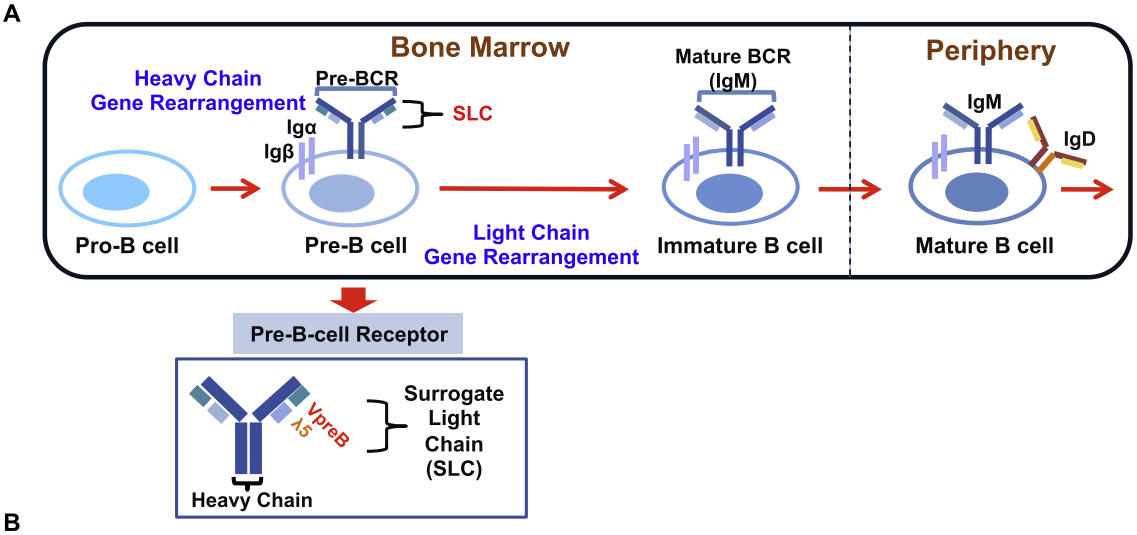

Fig. 1. Concept of the "common light chain". (A) During B cell development, approximately 50% of the newly rearranged heavy chains can bind to the surrogate light chain at the pre-B stage and differentiate into mature B cells [18]. Thus, the surrogate light chain might have a propensity to be the common light chain associating with most of the heavy chains in the periphery. (B) Comparison between human-derived conventional light chain sequence (PDB: 3PIQ, bottom) and surrogate light chain sequence (PDB: 2H32, top) after alignment. The identical amino acids are indicated at the top of the sequence. Non-Ig region, J region, and CDR3 are annotated in green, orange, and purple, respectively. The red arrow indicates the positively charged Arg in the middle of VpreB (PDB: 2H32). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Transfection and antibody protein expression

The antibody expressions were analyzed as described previously [8]. For details, see Supplementary Methods.

2.4. Immunoassays

See Supplementary Methods.

2.5. Western blot analysis

See Supplementary Methods.

2.6. In silico simulation

Homology modeling and docking were performed using MOE, and molecular dynamics simulation was run with NAMD [19–23]. Procheck was used for the evaluation of the constructed antibody models [24]. For details, see Supplementary Methods.

3. Results

3.1. Construction of VpreBλ5Cκ light chains as "common light chain" models

The rearrangement of IgH genes occurs before that of IgL genes, where the surrogate light chain (SLC) is associated with nascent IgH instead of IgL [18] (Fig. 1A). Here, sequence alignments were performed between SLC (the sequences from PDB: 2H32) and conventional λ1 light chain (the sequence from PDB: 3PIQ). The region similar to CDR3 in the SLC was composed of a portion of VpreB and λ5 close to the non-Ig region [25] (Fig. 1A and B). As shown in Fig. 2, we modified the different SLCs to form the VpreBλ5 light chains (see Materials and Methods for the details). Because the SLC gene sequences differ among various species, both human and mouse-derived VpreB1λ5 light chains (Hs_VpreB1λ5 and Mm_VpreB1λ5) were designed (Fig. 2B). At an early phase of assembly, Ig-μH also associates with VpreB3 and λ5 [26,27]. We compared the gene sequences between human-derived VpreB1 and VpreB3 and synthesized the Hs_VpreB3λ5 light chain gene (Fig. S1).

In our previous studies, the most significant IgH repertoires were found to be NP-specific IGHV1-72 (short for IGHV1-72/IGHD1-1/IGHJ2) and CGG-specific IGHV9-3 (short for IGHV9-3/IGHD1-1/IGHJ2) (Fig. S2) [8]. To make it consistent with the λ1 light chain already known to pair with IGHV1-72 [12], the positively charged arginine (Arg) in the middle of the new CDR-L3 was replaced with a tyrosine (Tyr) carrying no charge (R101Y, RtY) (Fig. 1B). Upon introducing *in silico* mutations of R101Y in Mm_VpreB1λ5Cκ and Hs_VpreB1λ5Cκ, we found that the Arg residues in the middle of CDR-L3 in both Mm_VpreB1λ5Cκ and Hs_VpreB1λ5Cκ decreased the affinity for 4-hydroxy-3-nitrophenylacetic acid (NPA) when paired with IGHV1-72Cγ

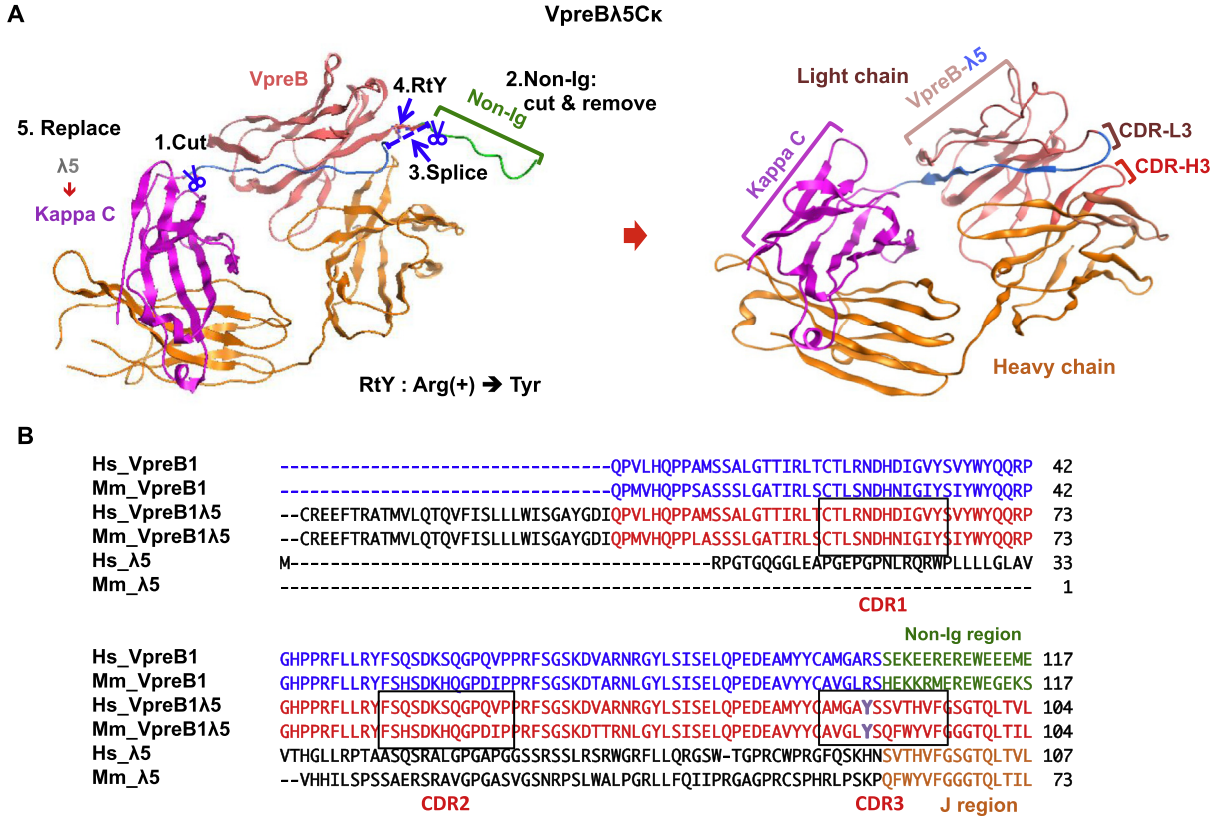

Fig. 2. Construction of recombinant VpreBλ5Cκ light chains. (A) Structural map of the genetically engineered light chain. VpreB (red), Cκ (purple), and heavy chain (orange) are shown. The "tail" of λ5 is highlighted in blue, and the non-Ig domain of VpreB is highlighted in green. The mutation from Arg to Tyr in CDR-L3 was annotated. The steps involved in genetic modification (left) are labeled in appropriate order: 1. Cut the "tail" of λ5; 2. Cut and remove the non-Ig region of VpreB; 3. Splice the "tail" of λ5 to VpreB; 4. Introduce the mutation of Arg101Tyr into VpreB; 5. Replace the λ5 with the human-derived Cκ. The intact antibody Fab (right) is also shown. In addition, all the variable regions of IgHs were connected with the human derived Igγ constant region as described in Materials and Methods. (B) Alignment of the partial amino acid sequences of VpreB1 and λ5 compared to that of those of VpreB1λ5 constructs derived from humans and mice. The variable regions of VpreB1λ5 and the corresponding regions in VpreB1 and λ5 are indicated in red, blue, and orange, respectively. The non-Ig region in VpreB is indicated in green. The mutation of Arg to Tyr is also highlighted in purple. The CDR regions in VpreB1λ5 constructs are annotated using the Kabat numbering scheme. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Fig. S3A); the replacement of Arg with Tyr also appeared to affect the heat stability of paired complexes (Fig. S3B).

The originally designed VpreB1λ5 light chains were connected to a human-derived Igκ constant region (Fig. 2A). We tested the replacement of the human-derived Igκ constant region with the human-derived Igλ5 constant region; however, the antibody affinities were significantly reduced by the replacement with λ5 constant region (Fig. S4).

3.2. Pairing of constructed light chain models to IgHs and their effect on antigen recognition of IgHs

The expression and secretion of paired IgH/IgL complexes were confirmed by Western blot of culture supernatants, showing that all light chain models (Mm_VpreB1λ5Cκ, Hs_VpreB1λ5Cκ, and Hs_VpreB3λ5Cκ) were paired with IGHV1-72Cγ and IGHV9-3Cγ heavy chains (Fig. 3A).

To assess the stereo-chemical qualities and antigen bindings of NP-specific IgHs paired with each light chain model (Mm_λ1Cκ, Mm_VpreB1λ5Cκ, and Hs_VpreB1λ5Cκ), *in silico* docking simulations were performed, and the optimal antigen binding patterns were analyzed (Fig. S5A). NP-specific IGHV1-72Cγ paired with Mm_λ1Cκ, Mm_VpreB1λ5Cκ, and Hs_VpreB1λ5Cκ showed the conserved antigen-binding site Arg50 in all three antibodies; however, Arg50 was found to bind to different oxygen molecules in IGHV1-72Cγ/Mm_λ1Cκ (Fig. S5B).

As shown in Fig. S2, the dominant CGG-specific IgH repertoires include the same V_H (IGHV9-3) and different D_H and J_H regions (Fig. S6), suggesting that the antigen specificity was not exerted by CDR-H3. To test the involvement of CDR-H1 and CDR-H2 in antigen binding, several mutations were introduced into t CDR-H1 and -H2 (Fig. S7A). As shown in Fig. S5B_a, Arg50 of IGHV1-72 was predicted to be one of the major residues to interact with NP. Consistently, the R50A mutant remarkably lost its antigen binding affinity (Fig. S7B). Similarly, all mutations in CDR-H1 and CDR-H2 of IGHV9-3 significantly induced a loss of binding affinities, suggesting that these regions were involved in the binding to CGG (Fig. S7B).

Among the light chain models constructed, Mm_VpreB1λ5Cκ properly maintained the binding of IGHV1-72 to NP antigen, although its binding capacity with the original light chain (Mm_λ1Cκ) was significantly higher; in addition, Mm_VpreB1λ5Cκ also maintained a high binding capacity of IGHV9-3 to CGG, whereas pairing with Mm_λ1Cκ decreased the binding capacity remarkably (Fig. 3B). It is noteworthy that conventional IgLs, Vκ_2ORB and Vκ_2A6I, considerably maintained the antigen binding of IGHV9-3 but not that of IGHV1-72 (Fig. 3B). We also detected the antigen binding by serial dilution of antibody concentration. In the NP-BSA binding test, the NP antigen was recognized at a concentration of 0.8 μg/mL when IGHV1-72 paired with Mm_VpreB1λ5Cκ and Mm_λ1Cκ, however it was recognized by other antibodies at higher concentrations; in the CGG-binding test, the CGG antigen was recognized at a concentration of 0.2 μg/mL when IGHV9-3 paired with Mm_VpreB1λ5Cκ, while it was recognized at higher concentrations when IGHV9-3 paired with Mm_λ1Cκ and Hs_VpreB3λ5Cκ (Fig. 3C). These results suggested that Mm_VpreB1λ5Cκ had the ability to pair with either IGHV1-72

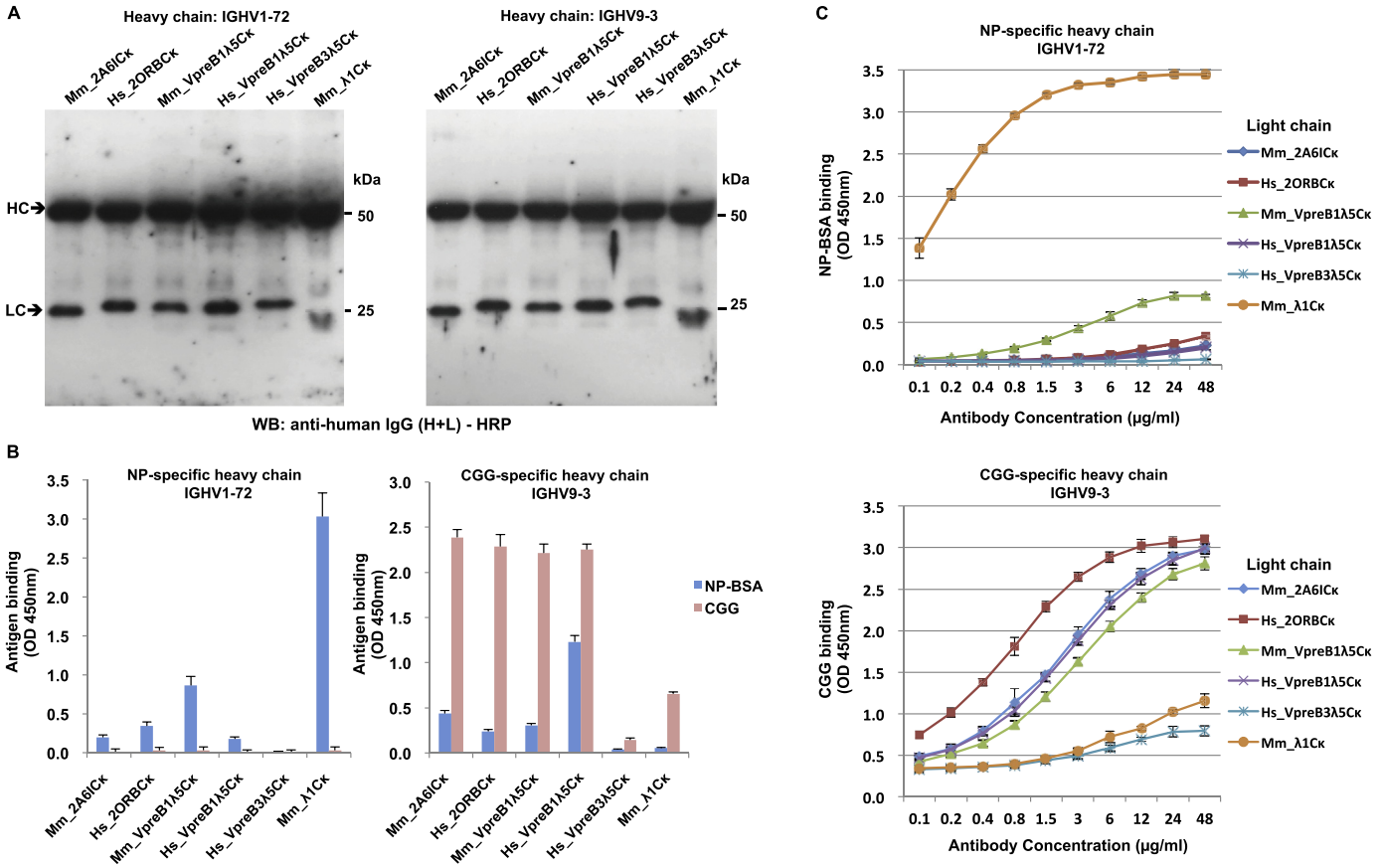

Fig. 3. Matching of different light chains and mouse-derived NP- and CGG-specific heavy chains. (A) Western blot analysis for the expression of recombinant antibodies. The heavy chain (top) and light chain (bottom) bands are shown. (B) Cell culture supernatants were tested for binding specificity and capacity to NP-BSA and CGG antigen. (C) Comparison of antigen binding affinity of different antibodies depending on the antibody concentration. Error bars indicate standard deviations of the means in three independent experiments.

or IGHV9-3 maintaining the antigen recognitions of IgHs.

3.3. Mm_VpreB1λ5Cκ can pair with several IgHs

To examine the ability of Mm_VpreB1λ5Cκ to match with different heavy chains, three additional NP-specific IgHs (IGHV1-53/D1-1/J2, IGHV6-3/D1-1/J2, and IGHV14-3/D1-1/J2) and three CGG-specific IgHs (IGHV1-69/D1-1/J2, IGHV1-55/D1-1/J3, and IGHV1-52/D2-4/J2) were chosen and then we tested the preservation of antigen-binding of each IgH (Fig. 4A and Fig. S2) [8].

Western blot analysis of the cell culture supernatants confirmed the expression and secretion of all antibodies (Fig. 4B). All the examined antibodies significantly bound to the corresponding original antigens maintaining the specificity and affinity, suggesting that Mm_VpreB1λ5Cκ is able to pair with a wide variety of IgHs maintaining their antigen specificities (Fig. 4C).

4. Discussion

In this study, we ascertained that the chimeric antibody consisting of a mouse-derived SLC variable region and a human-derived Igκ constant region could pair with several IgHs without losing the IgH antigen specificity and could maintain the antigen affinity of IgHs to a certain extent.

We previously analyzed NP- and CGG-specific IgH repertoires and their dynamics on a whole using NGS, and utilized the gene synthesis and expression system for the evaluation of important antibodies; however, the antigen binding seemed significantly affected by the chosen IgL [8]. We thought that if the antigen binding of both IgH and IgL can be separately analyzed, it would provide a novel experimental system to dissect the antigen binding dynamics of an antibody. We hypothesized that if the standard IgL, which can associate with a wide variety of IgH can be used, it would be possible to examine the molecular properties of IgHs without the bias of various inherent light chains. For the model of IgH/IgL pairing, it was reported that some IgLs can be shared by more than one IgH because IgL is less diverse than IgH [28,29].

The SLC would most likely exist as a “common light chain” for different IgHs because peripheral B cells use SLC for their IgH counterparts during their early development in the bone marrow [13–16]. Because the structures of the SLC remarkably show homology with the conventional IgL [25], we tried to transform the SLC molecules to construct “common light chain” models (Figs. 1 and 2). A similar work focusing on the variable domain of SLC has been reported, but the ability to pair with IgHs was not evaluated [30].

The Mm_VpreB1λ5Cκ molecule is associated with NP-specific IGHV1-72 IgH and CGG-specific IGHV9-3 IgH maintaining the antigen specificities and abilities, whereas Hs_VpreB1λ5Cκ and Hs_VpreB3λ5Cκ showed incomplete maintenance of antigen specificities. We tested the replacement of Cκ with $C_{\lambda 5}$ but the antigen binding properties were not improved (Fig. S4).

It was reported that, depending on the distances and interactions between residues on the V_H-V_L interface, antibodies can be classified into three different clusters (Mouse antibodies are grouped into both Cluster A and Cluster B, whereas human

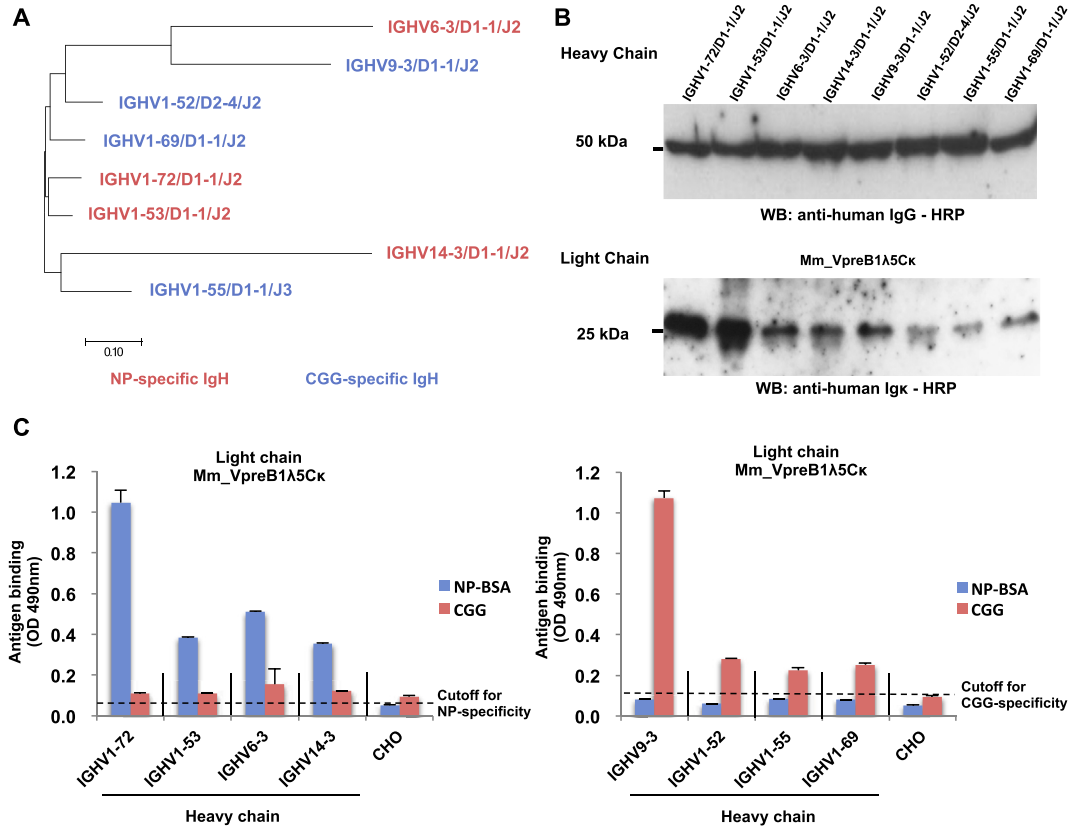

Fig. 4. Mm_VpreB1λ5Cκ matched with different NP-specific and CGG-specific heavy chains. (A) Phylogenetic tree for antigen-specific IgH repertoires: Red, NP-specific; Blue, CGG-specific. The tree was generated using the neighbor-joining (NJ) algorithm with the MEGA7 software (https://www.megasoftware.net). (B) Western blot analysis for the expression of recombinant antibodies. Heavy chain bands are at the top, and light chain bands are at the bottom. (C) Binding capacity of the recombinant antibodies to NP-BSA and CGG antigens. The cutoff was determined by the mean value plus standard deviation of CHO cell culture medium. The error bar indicates standard deviations of three independent experiments performed in triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

antibodies all belong to Cluster A) [31]. This implies that some mouse-derived IgLs might have wider heavy chain compatibility than human-derived IgLs. Two representative lgLs were chosen as the control to pair with both IGHV1-72 and IGHV9-3: the human-derived cluster A IgL (PDB: 2ORB.L) and mouse-derived cluster B IgL (PBD: 2A6I.L) (Fig. 3). Surprisingly, the conventional IgLs, mouse 2A6I.L and human 2ORB.L, preserved the antigen binding affinity of IGHV9-3; however, these conventional IgLs failed to preserve the antigen affinity of IGHV1-72 (Fig. 3B and C), suggesting significant differences in these IgH properties. As shown in Fig. S2B, IGHV9-3 used various IGHD genes in binding to CGG antigen, suggesting that the major antigen binding site is CDR-H1 or CDR-H2. In fact, mutations in CDR-H1 and CDR-H2 impaired CGG binding (Fig. S7). We speculate that the wide surface-to-surface interaction of an antigen and an antibody is less affected by the conformation skew induced by IgL species. In contrast, IGHV1-72 mainly used IGHD1-1 for NP-binding, suggesting that the major binding site is CDR-H3 in addition to R50 in CDR-H2 (Fig. S2A, S5B and S7B). In this case, the mode of antigen binding would be “lock and key” mode, which is prone to conformational skewing by the chosen IgL. In this context, Mm_VpreB1λ5Cκ was able to preserve the antigen recognitions of these distinct IgHs.

In this study, we constructed an engineered “common light chain” prototype, Mm_VpreB1λ5Cκ, which can associate with several IgHs (Fig. 4). It would be possible to improve the versatility of Mm_VpreB1λ5Cκ by *in silico* simulations and immunoassays to provide a novel antibody model in which the interference between IgH and IgL is properly controlled and insulated for optimized antigen recognition of artificial antibodies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.05.149.

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