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Deciphering antigen-responding antibody repertoires by using next-generation sequencing and confirming them through antibody-gene synthesis

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ABSTRACT

Vast diversity and high specificity of antigen recognition by antibodies are hallmarks of the acquired immune system. Although the molecular mechanisms that yield the extremely large antibody repertoires are precisely understood, comprehensive description of the global antibody repertoire generated in individual bodies has been hindered by the lack of powerful measures. To obtain holistic understanding of the antibody-repertoire space, we used next-generation sequencing (NGS) to analyze the deep profiles of naive and antigen-responding repertoires of the IgM, IgG1, and IgG2c classes formed in individual mice. The overall landscapes of naive IgM repertoires were almost the same for each mouse, whereas those of IgG1 and IgG2c differed considerably among naive individuals. Next, we immunized mice with a model antigen, nitrophenol (NP)-hapten linked to chicken γ-globulin (CGG) carrier, and compared the antigen-responding repertoires in individual mice. To extract the complete antigen response, we developed an intelligible method for detecting common components of antigen-responding repertoires. The major responding antibodies were IGHV1-72/IGHD1-1/IGHJ2 for NP-hapten and IGHV9-3/IGHD3-1/IGHJ2 for CGG-carrier protein. The antigen-binding specificities of the identified antibodies were confirmed through ELISA after antibody-gene synthesis and expression of the corresponding NGS reads. Thus, we deciphered antigen-responding antibody repertoires by inclusively analyzing the antibody-repertoire space generated in individual bodies by using NGS, which avoided inadvertent omission of key antibody repertoires.

1. Introduction

The antibody system's potency depends on its vast diversity and fine specificity of antigen recognition. The antibody-producing B-cell repertoire in an individual is generated by VDJ gene recombination in immunoglobulin gene loci [1,2], and its dimension is estimated to be $>10^{15}$ [3]. Immunization with an antigen triggers clonal expansion of antigen-specific B cells pre-formed in the immune system of individuals. To comprehensively understand the protective antibody response against invading pathogens without unconsciously omitting precious antibody repertoires, the overall B-cell repertoires and their dynamic changes in individual immune systems must be precisely described and analyzed. The B-cell repertoires generated in individuals had been regarded as a "black-box" because of the astronomical number of B-cell clones involved; however, the recent advent of next-generation sequencing (NGS) technology has led to a breakthrough in obtaining an overview of B-cell repertoires [4]. Whole antibody repertoires were first analyzed in zebrafish [5], and then in mice [6,7] and humans [6,8,9]. Although this method serves as a powerful tool for studying adaptive immune responses, the commonalities and uniqueness of

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antibody repertoires developed in individuals remain inadequately analyzed.

In this study, we obtained a global view of the common and unique features of antibody repertoires generated in individual mice by using the pyrosequencing technique. We also developed a simple and confirmative method to decipher antigen-responding antibody repertoires by comparing all aspects of B-cell repertoires of individual mice. The accuracy of the detected repertoires was confirmed through gene synthesis and protein expression of the antibody-sequence outputs from NGS.

2. Materials and methods

2.1. Mice and immunizations

We maintained 7–8-week-old C57BL/6J female mice (SLC Japan) in a specific-pathogen-free facility and immunized them intraperitoneally with 100 μg of either 4-hydroxy-3-nitrophenylacetyl $(NP)_{48}$-chicken γ-globulin (CGG) or CGG with alum-adjuvant, and 2 weeks later, collected the spleens for NGS analysis. All animal experiments were performed according to institutional guidelines and with the approval of the National Institute of Infectious Diseases Animal Care and Use Committee.

2.2. RNA preparation, cDNA synthesis, and 5′-RACE PCR amplification

Total RNA was extracted separately from each spleen by using a TRIzol Plus RNA Purification Kit (Thermo Fisher) (Fig. 1A and B), and 1–4 μg of the RNA was used for first-strand cDNA synthesis by SMARTer RACE cDNA Amplication Kit (Clontech) with oligo-dT-containing 5′-RACE CDS Primer A and SMARTer II A Oligonucleotide. Next, cDNAs were amplified by PCR in a 20 μl reaction mixture containing 0.5 μL of unpurified cDNA, 0.4U Phusion High-Fidelity DNA Polymerase, 200 μM each dNTP and 250 nM primers in 1xHF buffer. Universal forward primers of 5′-RACE containing Multiplex Identifier (MID) adaptors (MID9_NUP 5′-TAGTATCAGCAAGCAGTGGTATCAACGCAGAGT-3′, MID11_NUP 5′-TGATACGTCTAAGCAGTGGTATCAACGCAGAGT-3′, MID14_NUP 5′-CGAGAGATACAAGCAGTGGTATCAACGCAGAGT-3′) were used with reverse primers specific for immunoglobulin-constant-region-1 CμH1 (5′-CACCAGATTCTTATCAGACAGGGGGCTCTC-3′), Cγ1H1 (5′-

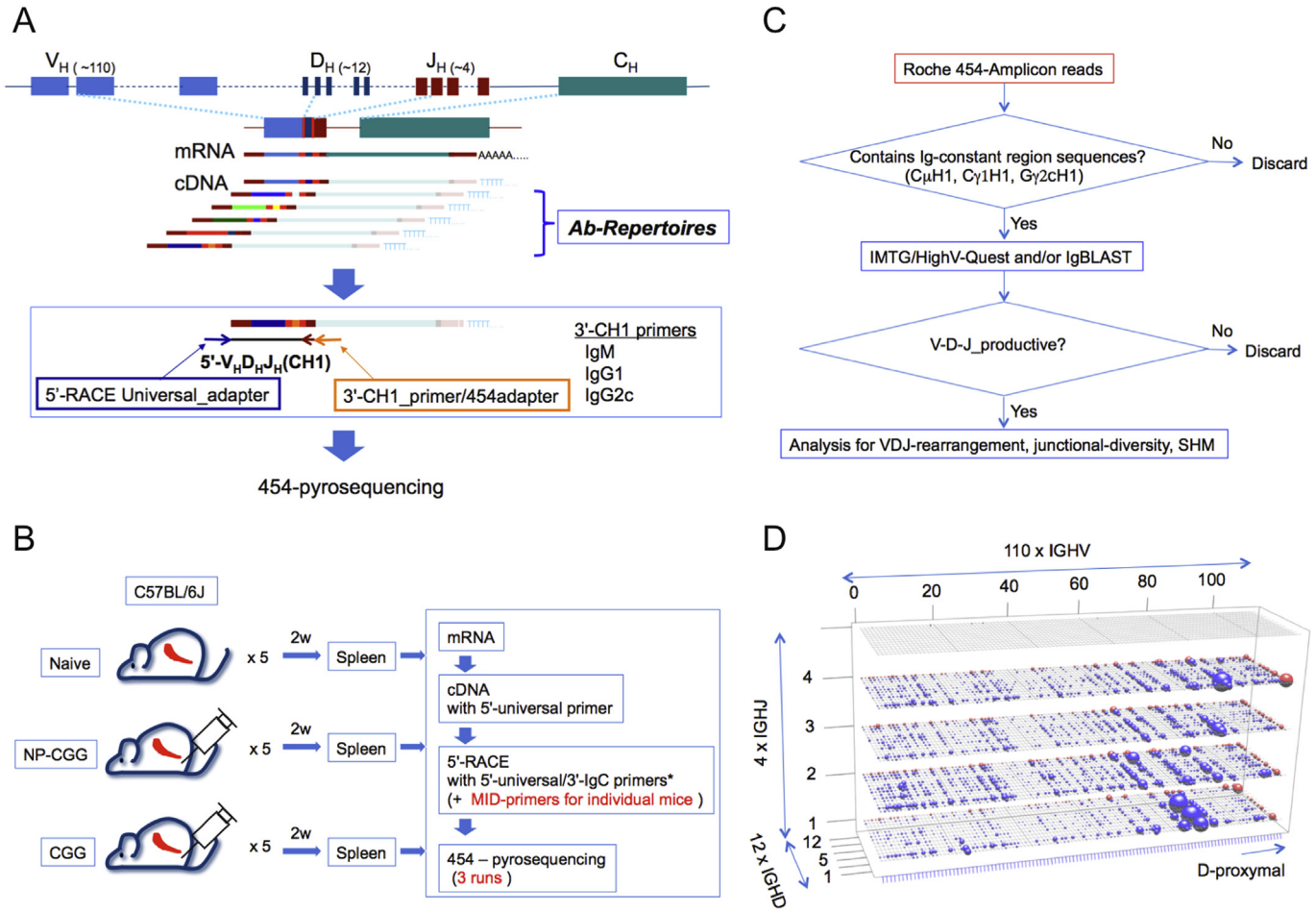

Fig. 1. Schematic of sequencing strategy, immunization protocol, data-processing flowchart, and data visualization for analyzing antibody repertoires in individual mice. (A) Spleen total RNA was reverse-transcribed and fragments containing V_H-D_H-J_H and partial CH1 were PCR-amplified using 5′-RACE universal primer and 3′-CH1 primer specific for CμH1, Cγ1H1, or Cγ2cH1. These PCR products representing IgM, IgG1, and IgG2c repertoires were equally mixed and pyrosequenced. (B) C57BL/6 mice (5 each) were not immunized (Naive group), immunized with NP-CGG (NP-CGG group), or immunized with CGG (CGG group), and after 2 weeks, spleen total RNA from each mouse was purified and pyrosequenced. (C) Amplicon reads obtained after pyrosequencing were processed as follows: (1) read sequences were translated in 6 reading-frames and checked for a defined sequence of CμH1, Cγ1H1, or Cγ2cH1; (2) sequences were examined using IMGT/HighV-Quest and IgBLAST; (3) purified sequences containing a productive VDJ junction were collected; and (4) these sequences were further analyzed. (D) To visualize the overall antibody-repertoire landscape, reads were arrayed on 3D-VDJ-plots in which the x-axis represents 110x IGHV genes and the y-axis represents 12x IGHD genes and 4x IGHJ genes. The gene order in the mesh is the same order as on the chromosome. Each sphere's volume represents the number of reads classified on the node. Red spheres: un-annotated V, D, and J genes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CATCCCAGGGTCACCATGGAGTTAGTTTGG-3′), or Cγ2cH1 (5′-GTACCTCCACACACAGGGGCCAGTGGATAG-3′) to amplify each isotype's repertoires through thermal cycling (98 °C for 30 s, 40 cycles of 98 °C for 10 s, 71 °C for 15 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min). The 600–800-bp PCR products were gel-purified using NucleoSpin Gel and PCR Clean-up kits (Macherey-Nagel). With this method, unlike with the use of degenerate 5′-Vh gene primers, the cDNAs of each antibody class were amplified evenly using the universal 5′-RACE primers. The purified amplicons were quantitated, and equal amounts of each antibody class (IgM, IgG1, IgG2c) from the same individual were pooled.

2.3. Pyrosequencing

The library was prepared using a GS Titanium Rapid Library Preparation Kit (Roche), as per the manufacturer's method. Library quality was assessed using an Agilent 2100 Bioanalyzer. Emulsion PCR and sequencing reactions were performed using a 454 GS Junior Titanium emPCR Kit and GS Junior Titanium Sequencing Kit (Roche).

2.4. Sequence assignment and visualization of VDJ repertoires

Raw reads from the 454-system were filtered for sequence quality by using the 454 Roche pipeline, and filter-passed reads were translated into amino acids in 6 reading-frames and checked for a signature sequence of Cμ H1 (SQSFP), Cγ1H1 (KTTPP), or Cγ2cH1 (KTTAP) [10]. The reads sorted into IgM, IgG1, and IgG2c groups were analyzed using IMGT/HighV-Quest [11] and standalone IgBLAST [12] to assign V-, D-, and J-gene segments (Fig. 1C). Sequences containing productive VDJ junctions were collected. To visualize the overall view of antibody repertoires, the reads were arrayed on a 3D mesh in which the x-axis represented 110 × IGHV genes and the y-axis represented 12 × IGHD and 4 × IGHJ genes (Fig. 1D); the gene order in the plot mesh is the same as on the chromosome [13], and is listed in Supplemental Method. Each discrete point ("node") in the figure represents a combination of V, D, and J, and the volume of the sphere assigned to a node indicates the number of reads (total read number was normalized to 1,000,000). The red spheres represent un-annotated V, D, and J genes, and the visualized image is called a "3D-VDJ-plot" (Fig. 1D). Data processing, including figure drawing, was performed by using in-house computer programs with Perl, and R [14]; the "rgl" package [15] of R was used for figure drawing.

2.5. Deciphering antigen-responding antibody repertoires

We developed a simple and confirmative method to reveal the antigen-responding antibody repertoires that commonly appeared in 5 mice after immunization. The 3D-VDJ-plot was drawn for each mouse in the naive, NP-CGG, and CGG groups, and the commonalities of each node of the 3D-VDJ-plot were tested thus:

(1) The read number on each node of the 3D-VDJ-plot was represented as

$$R_{vdj} \quad (1 \le v \le 111,\ 1 \le d \le 13,\ 1 \le j \le 5)$$

where R_{vdj} is the number of reads on each node, with v, d, and j subscripts indicating the IGHV, IGHD, and IGHJ numbers, respectively (each number includes the nodes for "un-annotated genes" in addition to genuine gene numbers). The 3D-VDJ-plot contains 7215 nodes in total.

(2) The presence of reads on each node among the 5 mice was represented as

$$N_k = \left[R^1_{vdj},\ R^2_{vdj},\ R^3_{vdj},\ R^4_{vdj},\ R^5_{vdj}\right] \quad (k = 0,\ 1 \ldots 7215)$$

where N_k is an array of read numbers of the 5 mice at each node, with the subscript k indicating the node number and superscripts 1–5 indicating the mouse numbers.

(3) The commonality—the concurrent occurrence of distinct VDJ reads in the 5 mice—was ranked from 1 to 5, depending on the numbers of nonzero in the elements of the array N_k. The means of the read number on each node of ranks were represented as

$$\begin{aligned}
{}^1M_k &= \text{mean1}\ N_k \quad (k = 0, 1 \ldots 7215)\\
{}^2M_k &= \text{mean2}\ N_k \quad (k = 0, 1 \ldots 7215)\\
{}^3M_k &= \text{mean3}\ N_k \quad (k = 0, 1 \ldots 7215)\\
{}^4M_k &= \text{mean4}\ N_k \quad (k = 0, 1 \ldots 7215)\\
{}^5M_k &= \text{mean5}\ N_k \quad (k = 0, 1 \ldots 7215)
\end{aligned}$$

where ${}^1M_k$ is the mean of the element of N_k in which one element is not 0, ${}^2M_k$ is the mean of the element of N_k in which 2 elements are not 0, and so on; ${}^5M_k$ represents the mean of the element of N_k in which 5 elements are not 0, i.e., the mean of the numbers of the VDJ read that was common to all 5 mice. The VDJ repertoires that were common to all 5 mice were visualized as a 3D-VDJ-plot by using ${}^5M_k$ data.

2.6. Multivariate analysis

Cluster analysis of VDJ profiles of individual mice was performed using N_k array data and "hclust" function of R-resource ("ward" method with "canberra" distance) [14]. Pearson's correlation coefficients of IGHV usage between individual mice were calculated using the array of IGHV-usage frequency of each mouse.

We developed a program to visualize changes in expression levels of the immunoglobulins featuring different VDJ combinations that appeared in the 5 individual mice. Consider that a set of amino acid sequences exists for an immunoglobulin type, with the sequences obtained under two different conditions, such as, in our case, from distinct individuals. The amino acid sequences derived from the two conditions were aligned, and based on the alignment, identical sequences, excluding the terminal gaps, were classified into groups. The longest sequence in each group was selected as the group's representative sequence, and the distance between every possible pair of the groups was calculated as the difference between the corresponding representative sequences. Next, a rooted dendrogram generated by using the UPGMA method with the distance was drawn as a circular tree. A line extending outside from each leaf indicates, by its length, the numbers of the sequences included in a group corresponding to the leaf. The two colors of a line correspond to the different conditions. The alignment was performed using MAFFT (option: FFT-NS-2) [16], and the dendrogram was generated as a postscript file by a program written using C-language.

2.7. Antibody-gene synthesis and expression

The VDJ sequences of detected antibody genes were selected from NGS-output reads, and the genes were synthesized and cloned into an antibody-expression vector in which human-IgG1

constant region and κ-light-chain constant region are used (Mammalian Power Express System, TOYOBO). The synthesized heavy-chain genes in pTAKN vector were inserted into pEHgammaX1.1 vector by using *Bsi*WI and *Nhe*I-HF sites. The synthesized light-chain genes in pTAKN vector were inserted into pEHkappaX2.2 vector by using *Bsi*WI and *Mlu*II sites. We used 3 types of light-chain V-genes (Vλ1, Vκ_2ORB, Vκ_2A6I) for the counterpart of heavy-chains [17]. CHO cells were transfected with the construct and screened using puromycin (10 μg/mL). Antibodies secreted into culture medium were tested using ELISA for binding to NP and CGG.

3. Results

3.1. Antibody-repertoire deep sequencing

To analyze antibody repertoires inclusively, we used high-throughput pyrosequencing. We analyzed immunoglobulin transcripts from entire spleens rather than from cell-sorted B-cell populations, because we aimed to obtain an overview of entire antibody repertoires in individual mice. Total RNA was extracted separately from the excised spleen of each mouse, reverse-transcribed with SMARTer-oligos, and PCR-amplified with 5′-RACE universal forward primer and reverse primers specific for the immunoglobulin constant region of IgM, IgG1, and IgG2c (Fig. 1A). In addition to 5 naive mice, we used 5 mice each immunized with a widely used model antigen, NP-hapten, and CGG-carrier (Fig. 1B). The raw-read sequences from the 454-sequencer were checked for quality and processed (Fig. 1C). This sequencing strategy yields data on mRNA-species amounts in the overall repertoires of antibody classes, which reflect the number and transcriptional activities of B cells in the immune system. One NGS run was used for mice of the same immunization status. From each run, 183,322, 157,498, and 131,047 reads were obtained (Supplemental Table S1). To visualize globally the VDJ-rearrangement profile in individual mice, the VDJ pattern was displayed on 3D-VDJ-plots, in which each sphere's volume represents the relative frequency of an antibody featuring a specific VDJ combination in overall B cells (Fig. 1D). Comparison of two technical replicates showed that the results were highly reproducible, indicating that our sequencing protocol and data-processing pipeline were stable and adequately sensitive for antibody-repertoire analysis (Supplemental Fig. S1).

3.2. Antibody repertoires of naive and immunized mice

Antibody repertoires of naive and immunized mice were analyzed by comparing the IgM, IgG1, and IgG2c sequences from 5 each naive mice (Supplemental Fig. S2), NP-CGG-immunized mice (Supplemental Fig. S3), and CGG-immunized mice (Supplemental Fig. S4). Cluster analysis of the profiles of 3D-VDJ-plots of these 15 mice revealed that the VDJ profiles of IgM were clustered separately from those of IgG1 and IgG2c (Fig. 2A). When the IGHV-usage profiles of IgM, IgG1, and IgG2c were compared for the 5 naive mice, a strong non-random bias in IGHV usage was observed in IgM repertoires but not IgG1 or IgG2c repertoires (Fig. 2B). The profiles of IGHV-usage frequency of naive IgM were highly correlated between individuals (mean Pearson's r = 0.644 ± 0.186; Supplemental Fig. S5). IGHV11-2, in particular, was highly expanded in the IgM repertoire in all mice (Fig. 2B, Supplemental Fig. S2). Conversely, IgG1 and IgG2c repertoires were not correlated between individual naive mice (mean Pearson's r = 0.153 ± 0.263 and 0.240 ± 0.213, respectively; Supplemental Fig. S5). However, after immunization, the IGHV usage of IgG1

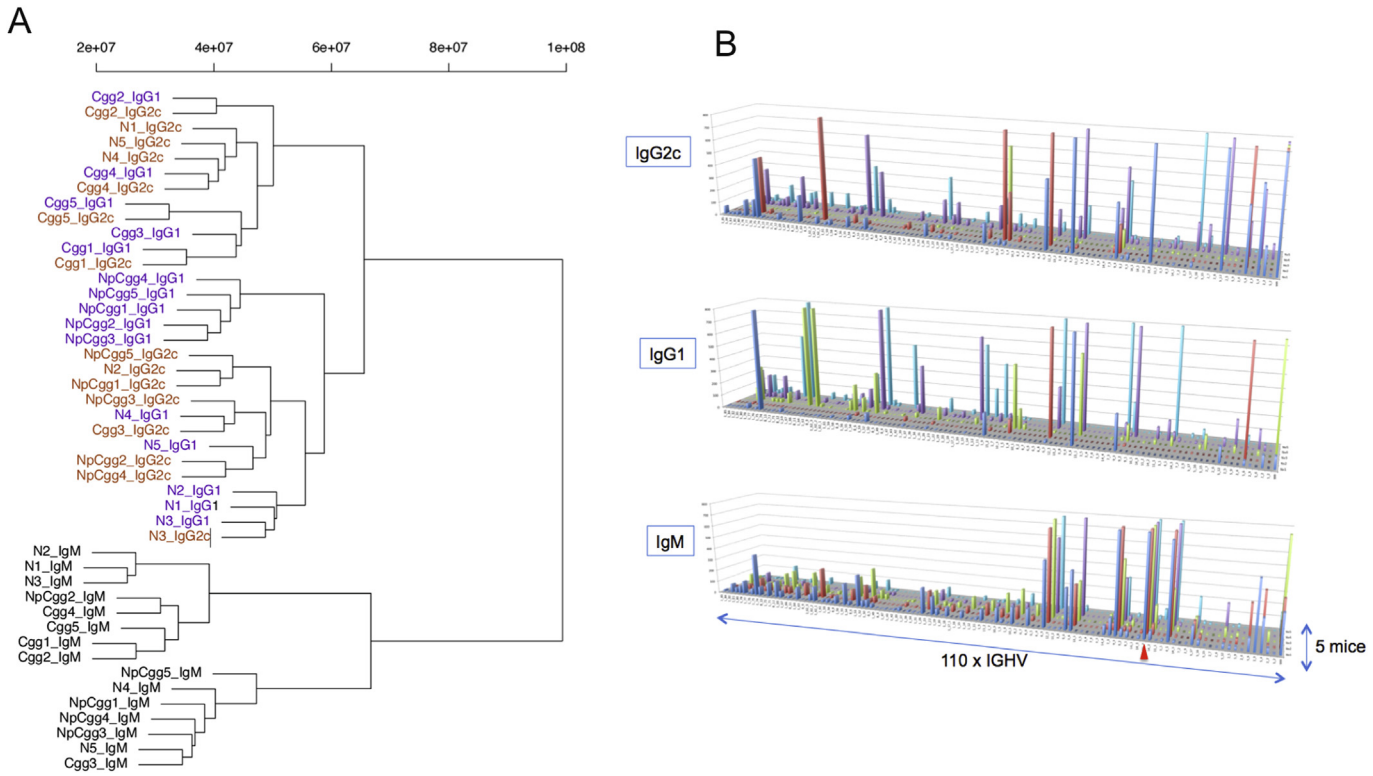

Fig. 2. Cluster analysis of VDJ profiles and usage frequencies of IGHV genes. (A) Cluster analysis was used for examining the profiles of 3D-VDJ-plots (i.e., the VDJ-combination frequency distributions). Similarities of VDJ profiles of IgM (black), IgG1 (blue), and IgG2c (red) were analyzed for naive mice (N1–5), NP-CGG-immunized mice (NpCgg1–5), and CGG-immunized mice (Cgg1–5). (B) Bar graphs showing usage frequencies of 110 IGHV genes in IgM (bottom), IgG1 (middle), and IgG2c (top) in 5 naive mice. Arrowhead: IGHV11-2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

repertoires was highly correlated in the 5 NP-CGG-immunized and 5 CGG-immunized mice (mean Pearson's r = 0.620 ± 0.203 and 0.843 ± 0.158; Supplemental Fig. S6), which suggests that antigen immunization induced selective amplification of a specific set of IGHV genes.

3.3. Deciphering antigen-responding repertories

To identify antigen-responding antibodies from the global view of antibody repertoires, we attempted to establish a method for detecting the major antibody repertoires shared by the 5 mice (biological replicates) of the same immune status (Supplemental Figs. S2, S3, S4). However, from the results in Figs. S2, S3, and S4, these antigen-responding antibody repertoires cannot be deciphered. Next, the method was applied for the IgG1 repertoires of naive, NP-CGG-immunized, and CGG-immunized groups (Fig. 3). When the commonalities of the reads on each node of the 3D-VDJ-plots were calculated (*Materials and methods*), the antigen-specific repertoire profiles were successfully revealed using this panning method (Fig. 3). Naive mice did not appear to share VDJ repertoires other than due to fortuitous co-occurrence, whereas VDJ profiles were strongly correlated in NP-CGG-immunized and CGG-immunized mice; this implies that immunization increased a specific set of VDJ profiles of mice with the same genetic background. The major IGHVs commonly observed in the biological replicates were IGHV1-72 and IGHV9-3 for the NP-CGG and CGG groups, respectively. Given that IGHV1-72, also known as Vh186.2, is a widely recognized anti-NP antibody in C57BL/6 mice [18,19], our method successfully detected the canonical antigen-specific repertoire. In the case of IGHV1-72 antibodies, IGHD usage was confined to IGHD1-1, whereas several IGHD genes, IGHD3-1 to 4-1, were inclusively used in the case of IGHV9-3 antibodies. To the best of our knowledge, IGHV9-3 is the first described CGG-specific antibody. We also detected minor repertoires that were common in the NP-CGG-immune and CGG-immune groups.

3.4. Sequence analyses of identified antibodies

To analyze the VDJ region of the translated NGS reads of the deciphered antigen-specific antibodies, multiple-sequence alignment was performed using MAFFT [16] (Supplemental Figs. S7 and S8). The phylogenic-tree analysis of the major NP-responding repertoire, IGHV1-72/IGHD1-1/IGHJ2, revealed that the sequences were highly similar in the 5 mice. At least 5 distinct VDJ-sequence combinations were commonly expressed in the 5 mice after immunization with the same antigen, which indicates that the same

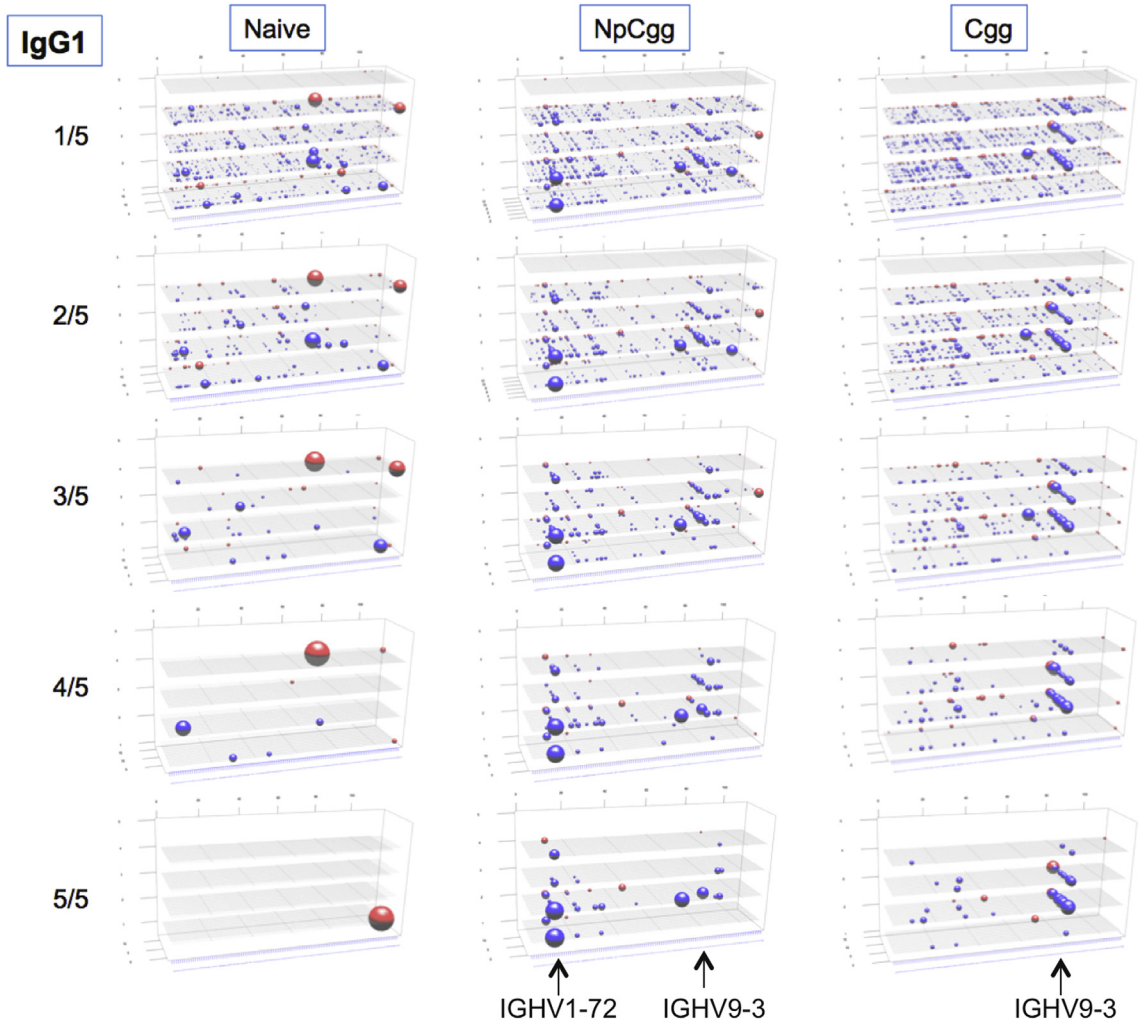

Fig. 3. Detection of antigen-responding antibodies from the global repertoire. IgG1 repertoires of naive, NP-CGG, and CGG groups were processed as described in *Materials and methods*. The 3 panels on top: summation of the 5 mice of each group; the 3 panels in the second row, 2/5: collection of reads that appeared at least in 2/5 mice; 3/5: reads that appeared in 3/5 mice; 4/5: reads that appeared in 4/5 mice; bottom row: reads that commonly appeared in the 5 mice. The major repertoires identified in the immunized groups, the IGHV1-72 and IGHV9-3 populations, are indicated.

antibody was frequently generated in different individuals (Fig. 4A). This analysis also yielded the profile of somatic hyper-mutation (SHM) development. Of the two types of high-affinity mutation, W33L-type and 95G-type, the 95G-type was reported to require SHM accumulation for affinity maturation [20]. Here, the 95G-type affinity maturation was still the minority as compared to W33L-type at 2 weeks after immunization (Fig. 4A). The dendrogram of the major CGG-responding repertoire, IGHV9-3/IGHD3-1~4-1/IGHJ2, also showed that the sequences were highly similar among the 5 mice (Fig. 4B).

3.5. Gene synthesis and expression of deciphered antibodies

Lastly, to confirm that the identified antigen-specific antibodies bind to the corresponding antigens, we synthesized the antibody genes from NGS sequences, expressed the proteins encoded by the genes, and performed ELISA to examine their antigen-binding ability. We arbitrarily selected one read each from IGHV1-72/IGHD1-1/IGHJ2 and IGHV9-3/IGHD3-1/IGHJ2 and synthesized the genes, which were cloned into an antibody-expression vector in which human-IgG1 constant region and κ-light chain are used. We used 3 types of light-chain V-genes (Vλ1, Vκ_2ORB, Vκ_2A6I) for the counterpart of heavy-chains [17]. The antibodies were expressed in CHO cells and the secreted antibodies were tested using ELISA for NP and CGG binding. The results confirmed the reactivity of the antibodies, although the heavy-chain binding capabilities were found to be affected by the counterpart light-chains (Fig. 4C).

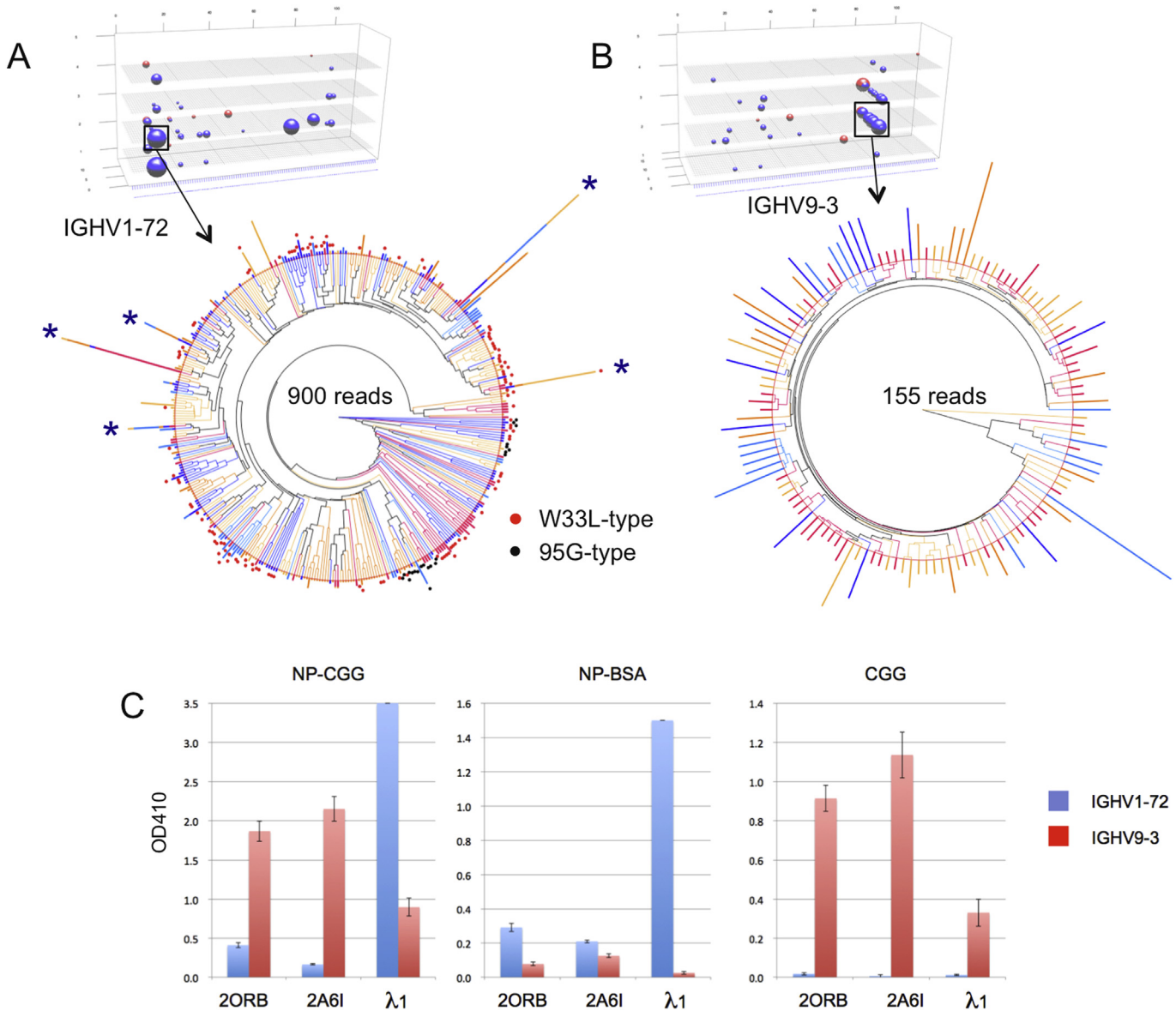

Fig. 4. Phylogenic-tree analysis of major antigen-responding antibodies, IGHV1-72 and IGHV9-3, and confirmation of antigen specificity through VDJ-gene synthesis and expression. (A) Sequences of the major NP-responding repertoire, IGHV1-72/IGHD1-1/IGHJ2, were translated into amino acid sequences and aligned for phylogenic-tree analysis. The 5 colors of the wheel tree represent the 5 individual mice. The length of bars radiating outside the wheel represents the number of reads. Asterisks: sequences found commonly in different individuals; red and black circles: high-affinity mutations, W33L-type and 95G-type, respectively. (B) Sequences of the major CGG-responding repertoire, IGHV9-3/IGHD3-1~4-1/IGHJ2, were translated into amino acid sequences and aligned for phylogenic-tree analysis. (C) Gene synthesis was performed for a randomly selected VDJ sequence from the IGHV1-72/IGHD1-1/IGHJ2 repertoire and the sequence was expressed with 3 representative V_L-domains of light-chain 2ORB, 2A6I, and λ1. Antibodies were expressed in CHO cells and culture supernatants were tested using ELISA for binding to NP-CGG, NP-BSA, and CGG. Data represent means ± S.D. of triplicate experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

We performed pyrosequencing on antibody RNAs amplified using the 5′-RACE method. With this method, in contrast to what occurs when using degenerate 5′-Vh gene primers, the mRNAs of each antibody class were amplified evenly with the universal 5′-RACE primers. Moreover, the use of reverse primers for the CH1 of antibody genes enabled profiling of specific immunoglobulin-class repertoires. This approach was highly favorable for both dissecting class-specific antibody responses and comparing naive and immunized repertoires.

Approximately 20% of the reads sequenced using the 454-system are generally considered to be ambiguous [21]. Although several correction methods can be used, 5%–10% remain ambiguous [4]. Therefore, we translated the sequences and filtered the raw reads that yielded long amino acid sequence containing the corresponding immunoglobulin constant regions (i.e., CμH1, Cγ1H1, Cγ2cH1).

The global expression patterns of antibody repertoires of individual naive mice revealed that the VDJ profile of IgM repertoires was more highly conserved than those of IgG1 and IgG2c. According to previous reports, VDJ combinations are highly stereotyped in immature zebrafish [22], and are also highly skewed in humans [6]. These non-random VDJ profiles were attributed to either preferential VDJ rearrangements or the selection over the antigens presented in the body. For example, IGHV11-2 was reported to be dominant in the fetal IgM repertoire [23]. This IGHV11-2 predominance in naive IgM is attributed to autoantibodies against the determinant on senescent erythrocytes [23]. IGHV11-2 was also the common major repertoire in our analysis of naive IgM.

In the aforementioned context, we found that antigen immunization focused the non-oriented repertoire to the antigen-specific repertoire in class-switched antibodies. We established a method to decipher the antigen-responding repertoires by extracting the commonalities in biological replicates of the same immune status. By applying this simple method to the VDJ repertoire of the 5 immunized mice, the common components of antigen-responding repertoires were successfully revealed.

We confirmed that the predicted heavy-chain repertoires bind to the immunizing antigen by synthesizing and expressing heavy-chain genes. In the expression vector, human IgG1 heavy-chain constant region and κ-light chain were used. However, antibodies expressed from this vector showed distinct antigen affinities when we used 3 types of canonical light-chain V-domain [17]. Thus, future studies must establish either a method for determining light-chain pairing to specific heavy chains, or a “universal light-chain” that can associate with diverse heavy chains without losing its antigen-binding properties.

We have established a simple prediction protocol for identifying antigen-responding antibody repertoires from a global repertoire in the body. By using the well-studied model antigen NP-CGG and CGG-carrier, we ascertained that the overall perspective of antibody dynamics against an immunizing antigen is explicitly descriptive in terms of the resolution of the NGS method. We should now potentially be able to examine the details of antibody-network dynamism, which would facilitate accelerated discovery of protective antibodies against newly emerging pathogens.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2017.04.054.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2017.04.054.

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