Immunoglobulin profiling identifies unique signatures in patients with Kawasaki disease during intravenous immunoglobulin treatment

Tai-Ming Ko1,2,3, Kazuma Kiyotani1, Jeng-Sheng Chang4,5, Jae-Hyun Park1, Poh Yin Yew1, Yuan-Tsong Chen2,6, Jer-Yuarn Wu2,7,* and Yusuke Nakamura1,*

1Department of Medicine, University of Chicago, Chicago, Illinois 60637, USA, 2Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan, 3Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung 404, Taiwan, 4Department of Pediatrics, China Medical University Hospital, Taichung 404, Taiwan, 5College of Medicine, China Medical University, Taichung 404, Taiwan, 6Department of Pediatrics, Duke University Medical Center, Durham, North Carolina 27710, USA and 7School of Chinese Medicine, China Medical University, Taichung 404, Taiwan

*To whom correspondence should be addressed at: Section of Hematology/Oncology, Department of Medicine, The University of Chicago, 900 E. 57th St., KCBD6130, Chicago, IL 60637, USA. Tel: +1 7738341405; Email: ynakamura@bsd.uchicago.edu (Y.N.); Institute of Biomedical Sciences, Academia Sinica, 128, Academia Road, Section 2, Nankang, Taipei 11529, Taiwan. Tel: +886 227899075; Email: jywu@ibms.sinica.edu.tw (J.-Y.W.)

Abstract

Identifying the causes of high fever syndromes such as Kawasaki disease (KD) remains challenging. To investigate pathogen exposure signatures in suspected pathogen-mediated diseases such as KD, we performed immunoglobulin (Ig) profiling using a next-generation sequencing method. After intravenous Ig (IVIG) treatment, we observed disappearance of clonally expanded IgM clonotypes, which were dominantly observed in acute-phase patients. The complementary-determining region 3 (CDR3) sequences of dominant IgM clonotypes in acute-phase patients were commonly observed in other Ig isotypes. In acute-phase KD patients, we identified 32 unique IgM CDR3 clonotypes shared in three or more cases. Furthermore, before the IVIG treatment, the sums of dominant IgM clonotypes in IVIG-resistant KD patients were significantly higher than those of IVIG-sensitive KD patients. Collectively, we demonstrate a novel approach for identifying certain Ig clonotypes for potentially interacting with pathogens involved in KD; this approach could be applied for a wide variety of fever-causing diseases of unknown origin.

Introduction

Fever of unknown origin (FUO) is defined as a syndrome of fever that does not recover spontaneously; its causes are, in general, difficult to identify despite the use of various diagnostic approaches (1). FUO in clinical practice causes a diagnostic dilemma for pediatricians because it is not easy to distinguish a fever with benign prognosis from that having a life-threatening risk (2). It is suspected that FUOs are caused or triggered by...
infections of bacteria, viruses, parasites or fungi (3). Patients with FUO often need to undergo many laboratory tests, as well as antimicrobial therapies; however, sometimes they experience the delay of receiving an appropriate treatment (4).

To overcome the difficulties in identifying a causative pathogen(s), we thought that the characterization of the disease-related signatures on immune receptor repertoires could be a useful approach in identifying a possible pathogen(s). Our immune system uses receptor proteins, which are expressed on the surface of the B and T lymphocytes, and can recognize the non-self-origin of peptides that are derived from pathogens or cancerspecific somatic mutations. For example, the B-cell receptor (BCR), which consists of the immunoglobulin heavy and light chains, possesses extremely high levels of diversity to recognize a wide variety of non-self-antigens. Functional BCRs are generated by the combination of exons in the BCR genes and nucleotide insertion/deletions during the recombination process as well as somatic hypermutation events (5). Through these mechanisms, lymphocytes maintain the repertoire with an extremely high number of unique receptors to prepare against a huge range of pathogens present in our environment (6,7).

Kawasaki disease (KD), a type of FUO, is a leading cause of acquired heart diseases in children (8). The cause of this disease is currently unknown; however, clinical and epidemiological features strongly suggest that infectious agents are likely to trigger KD and that genetic predispositions may play a role in its etiology (9,10). A possibility of B-cell involvement in KD was indicated by identifying the associations of SNPs clustered in IGHV (encoding the immunoglobulin heavy chain variable regions) with the risk of KD (11). Moreover, two comprehensive genome-wide association studies in different ethnicities emphasized the pathogenic role of B cells in KD because BLK (encoding B-lymphoid tyrosine kinase), which is selectively expressed in B cells, is the most significantly-associated genetic locus for KD (12,13). In this study, we hypothesized that certain unique features of the BCR repertoire could possibly be associated with this fever condition of an unknown origin and performed BCR sequencing of peripheral blood samples of pre- and post-IVIG treatment in 40 KD patients.

Results

Clonal expansion of immunoglobulin M (IgM) in KD
To investigate the possibility that some pathogens in KD patients might have induced IgM responses, we first performed an IgM repertoire analysis using RNAs isolated from the peripheral blood samples of KD patients. We then compared the IgM clonotypes at two time-points of KD patients who responded well to a single IVIG treatment (IVIG-sensitive cases); one at an acute phase (before IVIG treatment) and the other after recovery (2 months after IVIG treatment and no KD clinical symptoms were observed). In the first discovery group (n = 6) of the IVIG-sensitive KD cases (Supplementary Material, Fig. S1), we found that the diversity index of the IgM repertoire at the acute phase was significantly lower than that at the recovery phase (Fig. 1A), indicating expansion of B cells with certain BCR clonotypes at the acute phase.

A subset of KD patients did not achieve the full recovery and retained some KD symptoms by the single IVIG treatment (these patients were defined as IVIG-resistant cases). For such cases, we also performed an IgM repertoire analysis at three time-points, before the treatment as well as after the first and second IVIG treatments. In the IVIG-resistant KD patients (n = 6), the diversity of the IgM repertoire after the first treatment was still much lower than that observed in the IVIG-sensitive cases. However, the diversity of the IgM repertoire after the second treatment increased considerably, a condition similar to that condition after the single treatment of the IVIG-sensitive cases (Fig. 1B and C).

To further validate this infectious-disease-like IgM repertoire pattern, 24 IVIG-sensitive KD patients were additionally recruited for the replication study. In a combined analysis of 30 patients in the discovery and replication groups, we confirmed that the diversity of the IgM clonotypes in the acute stage was significantly lower than that in the recovery stage (Fig. 1D). Since individual patients had distinct IgM repertoires, we examined in detail the dynamic changes of activated B cells in these patients by comparing the most dominant IgM CDR3 clonotypes between the acute and recovery phases. The dominant IgM CDR3 clonotypes were defined as the ones with a frequency of 0.1% or higher in the all V–J–C mapped sequence reads. Among the 30 IVIG-sensitive cases, the dominant IgM CDR3 clonotypes observed before the treatment almost completely disappeared after the single IVIG treatment in 28 cases (Supplementary Material, Fig. S2).

Characterization of KD-associated CDR3 clonotypes
Upon the activation of B cells, the immunoglobulin heavy chain genes are known to rapidly undergo the process of class-switch recombination (CSR), which alters the antibody expression profile of B cells from IgM to other isotypes such as IgG and IgA (14). CSR enhances the ability of antibodies to eliminate a pathogen(s) by the humoral immune response (15). To examine the possibility that B cells with the high abundance were activated through exposures to some pathogens in the process of developing KD, we also performed sequencing of IgA and IgG, and compared their sequences with IgM sequences. The dominant CDR3 clonotypes of IgM in acute-phase KD patients were also frequently observed in IgG and IgA sequences in an acute phase of each of these patients (Table 1). Interestingly, the IgA- or IgG-clonotypes that had identical CDR3 sequences to the dominant IgM clonotypes also decreased significantly at the recovery phase (Table 1) (Paired t test; IgA, \( P = 1.5 \times 10^{-5} \); IgG, \( P = 6.6 \times 10^{-4} \)). To further examine whether KD patients may have common clonotypes that might recognize same antigens derived from same pathogens, we analyzed IgM CDR3 sequences of the frequency of 0.01% or higher among all IVIG-sensitive KD patients and identified 32 clonotypes that are present commonly in three or more cases (Table 2; Supplementary Material, Table S1); 23 unique IgM CDR3 sequences were not in any age-matched ten febrile non-KD individuals (Table 2). Multiple sequence alignment for these common IgM clonotypes in KD patients was analyzed (Supplementary Material, Table S2).

Clonotypes analysis in the IVIG-sensitive and IVIG-resistant cases
To examine whether there were any differences in BCR repertoires between the IVIG-sensitive and IVIG-resistant KD patients, we compared the sum of frequencies of dominant IgM CDR3 clonotypes (0.1% or higher) in these two groups at an acute phase (before IVIG treatment). The sum was significantly higher in the IVIG-resistant group (n = 10, we added four cases as a verification set) than that in the IVIG-sensitive group (n = 30) (Fig. 2A). To investigate a possibility of the sum of dominant CDR3 frequencies at an acute phase as a biomarker for predicting the response to the IVIG treatment, receiver-operating characteristic (ROC) curve analyses were performed. The sum of
frequencies of dominant IgM CDR3 clonotypes at an acute phase showed a very high area under the curve (AUC) value of 0.91 (Fig. 2B) when IVIG-resistant KD patients (n = 10) were considered the case group and IVIG-sensitive KD patients (n = 30) were considered the control group. We also compared the sum of frequencies of dominant IgM CDR3 clonotypes (0.01% or higher) in these two groups at an acute stage (before IVIG treatment), the AUC value was 0.89 (Supplementary Material, Fig. S3).

To further examine whether the IVIG-resistant KD patients have common BCR clonotypes that may recognize the same pathogen antigen(s), we performed overlap analysis for clonotypes with the frequency of 0.01% or higher and found 23 clonotypes to be present commonly in 2 or 3 of the 10 IVIG-resistant cases. Among these 23 clonotypes, 7 clonotypes were also found in some patients from the IVIG-sensitive group, while these sequences were not present from the non-KD control group (n = 10) (Supplementary Material, Table S3). It is notable that 10 clonotypes found commonly in IVIG-resistant patients were not found in any of the 30 IVIG-sensitive KD cases or 10 non-KD febrile controls (Supplementary Material, Table S3).

**Discussion**

It has been a big challenge to identify the cause(s) of fevers with unknown origins such as KD (9). Despite long-term clinical and basic investigations, it is still difficult to conclude whether KD is caused by infections or by unknown factors that can cause dysfunctions in our immune system. Although transcriptomic and proteomic profiling methods were applied to uncover the molecular characteristics of KD (16–19), the knowledge obtained through these approaches was quite limited for understanding the etiology of KD. In addition, because of a very broad spectrum of possible pathogens that might be related to the etiology of KD, a comprehensive screening for pathogens in KD could also be practically very difficult. Hence, we attempted to apply an alternative approach, the detailed immune repertoire analysis through deep sequencing of immunoglobulin transcripts, which may lead to the identification of the interaction between pathogens and their hosts in the process of disease development and progression (20–22).

In this study, we performed a comprehensive sequencing analysis of the transcripts of three immunoglobulin isotypes, IgM, IgG and IgA, using peripheral blood samples before and after the IVIG treatment of 40 KD patients, of which 30 were IVIG-sensitive cases (full recovery after a single IVIG treatment), and 10 were IVIG-resistant cases (full recovery after multiple IVIG treatments). We found that the immune repertoire in KD patients represented infection-like patterns, such as the very low diversity index of the IgM clonotypes in the acute phase, and the presence of certain dominant IgM clonotypes, most of which completely disappeared from the peripheral blood after the IVIG treatment(s). Moreover, we found some evidence indicating that the dominant sequences may undergo CSR, to produce pathogen-reacting antibodies through the affinity...
maturation process. Through the overlapping-clone analysis, we found certain clonotypes that were specific to patients with KD, or those that might be associated with responses to the IVIG treatment; however, a further validation using a larger set of KD patients (including IVIG-sensitive and IVIG-resistant cases) and febrile controls is warranted in the future. These results might provide critical clues or information that may be helpful to elucidate host-pathogen(s) interactions. Particularly, it is noteworthy that the sums of the frequencies of the dominantly detected IgM clonotypes were highly correlated with the response to the IVIG treatment, implying that the frequencies of dominant IgM clonotypes might be a good predictor for identifying IVIG-resistant patients.

Table 1. Frequencies of total IgG or IgA clonotypes shared with identical CDR3 sequences of dominant IgM clonotypes in acute-phase (before IVIG) IVIG-sensitive KD patients

| KD No. | Sum of the frequency of dominant clonotypes |
|--------|-------------------------------------------|
|        | Dominant IgM (before IVIG) (%) | Shared$^a$ IgG (before IVIG) (%) | Shared$^a$ IgG (2 months after IVIG) (%) | Shared$^a$ IgA (before IVIG) (%) | Shared$^a$ IgA (2 months after IVIG) (%) |
| 1      | 1.4 | 4.2 | 0.0 | 0.3 | 0.0 |
| 2      | 6.1 | 1.3 | 0.1 | 1.8 | 0.1 |
| 3      | 3.0 | 3.1 | 0.0 | 0.9 | 0.0 |
| 4      | 2.3 | 0.3 | 0.2 | 0.3 | 0.1 |
| 5      | 5.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| 6      | 17.3 | 1.5 | 0.0 | 1.6 | 0.0 |
| 7      | 15.3 | 2.0 | 0.0 | 0.7 | 0.0 |
| 8      | 0.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| 9      | 1.5 | 0.0 | 0.0 | 0.2 | 0.0 |
| 10     | 4.9 | 0.2 | 0.0 | 0.3 | 0.0 |
| 11     | 3.3 | 2.9 | 1.2 | 4.0 | 1.0 |
| 12     | 1.7 | 0.1 | 0.0 | 0.3 | 0.0 |
| 13     | 3.5 | 2.7 | 0.0 | 4.8 | 0.0 |
| 14     | 8.7 | 3.0 | 0.0 | 5.4 | 0.0 |
| 15     | 1.4 | 0.0 | 0.0 | 0.5 | 0.0 |
| 16     | 10.2 | 7.5 | 0.0 | 10.4 | 0.0 |
| 17     | 0.3 | 0.0 | 0.0 | 0.1 | 0.0 |
| 18     | 5.0 | 1.5 | 0.0 | 2.4 | 0.0 |
| 19     | 2.9 | 0.4 | 0.6 | 3.0 | 0.0 |
| 20     | 0.4 | 0.6 | 0.0 | 0.4 | 0.0 |
| 21     | 3.5 | 1.2 | 0.0 | 0.5 | 0.0 |
| 22     | 10.3 | 10.2 | 0.1 | 4.5 | 0.0 |
| 23     | 2.2 | 0.8 | 0.0 | 1.0 | 0.0 |
| 24     | 4.3 | 0.3 | 0.0 | 0.3 | 0.0 |
| 25     | 3.4 | 0.2 | 0.0 | 0.2 | 0.0 |
| 26     | 1.4 | 0.2 | 0.0 | 0.2 | 0.0 |
| 27     | 0.8 | 0.1 | 0.0 | 0.0 | 0.0 |
| 28     | 0.8 | 0.1 | 0.0 | 0.0 | 0.0 |
| 29     | 1.6 | 0.4 | 0.0 | 2.3 | 0.0 |
| 30     | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 |

$^a$‘Shared’ is defined as the same CDR3 clonotypes were observed commonly between IgM (before IVIG) and IgG or between IgM (before IVIG) and IgA. Dominant CDR3 clonotypes were defined as one with the frequency of 0.1% or higher.

Pathogen load may also contribute to the response of the IVIG treatment. The reason why ~10–20% of KD patients are required to receive multiple IVIG treatments for the full recovery is not well understood (25). In addition, in the clinical point of view, it is extremely important to identify or predict high-risk patients who may need multiple IVIG treatments. In this study, we found differences in IgM clonal expansion patterns between the IVIG-sensitive and IVIG-resistant groups. Since the expansion of certain clonotypes (with a frequency of 0.1% or higher) may be correlated with the pathogen load, our result implies that IVIG-resistant patients may have a higher pathogen load and stronger clonal expansion of B cells. In addition, the sum of the frequencies of dominant IgM clonotypes could potentially be a useful biomarker to identify IVIG-resistant patients. We also need to consider the possibility that some germline genetic variations may be associated with the extent of the clonal expansion of B cells.
The antibody repertoire is likely to reflect, in part, the history of pathogenic exposures (26). In cases for which the causative pathogens have not been identified, such as an FUO, it is clinically very important to monitor the dynamic changes of immune signatures through BCR/TCR analysis to better understand how our immune system responds to various infections and how autoimmune-like symptoms occur. Here, we quantified the changes in the BCR repertoire in patients with KD. We observed that most of the dominantly expanded IgM clonotypes quickly disappeared after the IVIG treatment across all KD patients although some patients needed multiple infusions. This suggests that KD represents an infection-like pattern in the BCR repertoire. In addition, some common clonotypes found in this study may provide valuable information that might lead to the identification of the causative agents of KD, including pathogens.

Finally, we found a unique feature of the IVIG-resistant group, which could be a potential biomarker for predicting the patients’ response to IVIG. Collectively, a comprehensive analysis of the immunoglobulin profile would serve as a novel approach to identify potential KD-causing pathogens, and could be applicable to a wide variety of fevers of unknown origin.

Materials and Methods

Patient groups

We enrolled 40 KD patients included 30 IVIG-sensitive patients (discovery group, n = 6; replication group, n = 24) and 10 IVIG-resistant patients (discovery group, n = 6; replication group, n = 4) (Supplementary Material, Fig. S1). KD was diagnosed with the 2004 and 2017 American Heart Association statement (25-27). Briefly, diagnosis of KD was based on the definition included fever (body temperature exceeding 38°C), accompanied by the presence of at least four of the following five findings: changes in the lips and oral cavity, bilateral conjunctival injection, non-purulent cervical lymphadenopathy, polymorphous exanthema, and changes in the extremities. Intraavenous immunoglobulin resistance was defined as persistent or recrudescence fever (body temperature exceeding 38°C) at least 48 h but not longer than 7 days after completion of the first IVIG infusion. We also enrolled 10 febrile pediatric patients with a fever and clinical features suggestive of KD (Supplementary Material, Table S4). Two echocardiographic examinations were performed during the acute stage of KD and two months after the onset of symptoms. All patients were recruited at the China Medical University Hospital Medical Center, Taichung, Taiwan. In the discovery group, samples were collected in the acute stage (Stage 1, defined as the stage before IVIG-treatment), post-first-IVIG stage (Stage 1, within a week after first-IVIG treatment and between first- and second-IVIG treatment) and recovery stage (Stage 3, defined as 2 months after IVIG-treatment and without any KD clinical presentations). In the replication group, samples were collected in the acute stage (Stage 1, defined as the stage before IVIG-treatment) and recovery stage (Stage 3, defined as Stage 2 months after IVIG-treatment).

Library preparation and immunoglobulin sequences analysis

About 2 ml of whole blood were collected in PAXgene Blood RNA collection tubes (Qiagen). Total RNA was extracted and purified per the manufacturer’s instructions (PAXgene Blood RNA Kit, Qiagen). Sequencing libraries of BCR were prepared using protocol described previously (28). Up to 300 ng of total RNA were used for cDNA synthesis, and a common adapter was added to the 5’ end of cDNA. PCR was designed to separately amplify different isotypes including IgM, IgG and IgA, using a forward primer designed on the common adapter and reverse primers corresponding to the C regions of each BCR isotype (Supplementary Material, Table S5). The first PCR protocol was as follows: 94°C for 3 min, followed by 20 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min. Illumina sequence adapters with barcode sequences from Nextera XT Index kit (Illumina) was then introduced to each sample to generate multiplexed sequencing libraries. This pooled library was sequenced by 300-bp paired-end reads on the Illumina MiSeq platform (Illumina), using MiSeq Reagent v3 600-cycles kit (Illumina). Immunoglobulin sequencing data were analyzed by Bcrip software (28). Briefly, sequence reads were mapped to the V, J and C reference sequences of each BCR isotype obtained from IMGT/GENE-DB (29,30) (www.imgt.org) with the Bowtie2 aligner (Version 2.1.0) (31). A CDR3 was defined by identifying the second conserved cysteine encoded in the 3’ portion of the V segment and the conserved phenylalanine or tryptophan encoded in the 5’ portion of the J segment.

Table 2. Common IgM clonotypes in acute-phase (before IVIG) IVIG-sensitive KD patients

| Common IgM clonotypes | KD patients (n = 30) (%) | Febrile controls (n = 10) (%) |
|-----------------------|------------------------|-----------------------------|
| CARDYYGYMDVW          | 30                     | 10                          |
| CARSDWFDPW            | 20                     | 0                           |
| CARHDWFDPW            | 17                     | 20                          |
| CARAGGYGYMDVW         | 13                     | 0                           |
| CARAGNYYYMDVW         | 13                     | 0                           |
| CARVDDYW              | 13                     | 0                           |
| CASKDFDWPW            | 13                     | 0                           |
| CARDGYYYYMDVW         | 10                     | 0                           |
| CARDGSSGWFDYPW        | 10                     | 0                           |
| CARDVSGSLDYW          | 10                     | 0                           |
| CARDDWFDYPW           | 10                     | 0                           |
| CARDGYYMDVW           | 10                     | 0                           |
| CARAGSYFDYW           | 10                     | 20                          |
| CARDDYYYMDVW          | 10                     | 0                           |
| CARGLYFYFDYW          | 10                     | 0                           |
| CARAGSFQFDYW          | 10                     | 10                          |
| CARDGYNWDYPW          | 10                     | 0                           |
| CGKDSIPGMDVW          | 10                     | 0                           |
| CVRGNYVFDPW           | 10                     | 10                          |
| CTTDPHYW              | 10                     | 0                           |
| CTTDPHYW              | 10                     | 0                           |
| CARAGYFDRYW           | 10                     | 10                          |
| CARGRFDYW             | 10                     | 0                           |
| CARPLRTGNWFDPW        | 10                     | 0                           |

Statistical analysis

The immunoglobulin diversity was calculated using the inverse Simpson’s diversity index formula. The inverse Simpson’s
diversity index = \sum_{i=1}^{n} \frac{\left( \frac{1}{K_i} \right)}{N}

where K is the total number of clonotypes, ni is the number of the ith clonotype sequence and N is the total number of sequences for which each clonotype is determined. Student’s t-test was used to compare the inverse Simpson’s diversity index and frequencies of clonotypes. Paired t test was used to compare the frequency (Fq) of total shared dominant clonotypes in Table 1. All statistical tests were conducted using Prism software, version 6.0 (GraphPad). In all statistical tests, P value of <0.05 was considered to be statistically significant. The ROC curve plots sensitivity and 1-specificity and provides a summary of sensitivity and specificity across a range of cutoff points for a continuous predictor. The optimal cutoff value of the sum of maximum sensitivity and specificity was determined as the sum of its maximum sensitivity and specificity.

**Supplementary Material**

Supplementary Material is available at HMG online.

**Acknowledgements**

The super-computing resource was provided by Human Genome Center, the Institute of Medical Science, the University of Tokyo for developing the algorithm of BCR repertoire analysis, Bcrip, and helpful support in data management. We thank doctors in China Medical University Hospital in Taichung, Taiwan for their contributions in recruiting patients with KD. We gratefully acknowledge the support of Ministry of Science and Technology (105-2314-B-001-009) and the members of Translational Resource Center for Genomic Medicine (TRC) of National Research Program for Biopharmaceuticals (NRPB) and the National Center for Genome Medicine (NCGM) of National Core Facility Program for Biotechnology (NCFPB), Ministry of Science and Technology, at Academia Sinica for their support in subject recruitment and data analysis. Funding to pay the Open Access publication charges for this article was provided by my grant from the university.

Conflict of Interest statement. None declared.

**References**

1. Rigante, D. and Esposito, S. (2013) A roadmap for fever of unknown origin in children. Int. J. Immunopathol. Pharmacol., 26, 315–326.
2. Mourad, O., PaIa, V. and Detsky, A.S. (2003) A comprehensive evidence-based approach to fever of unknown origin. Arch. Intern. Med., 163, 545–551.
3. McGregor, A.C. and Moore, D.A. (2015) Infectious causes of fever of unknown origin. Clin. Med. (Lond.), 15, 285–287.
4. Knockaert, D.C., Vanderschueren, S. and Blockmans, D. (2003) Fever of unknown origin in adults: 40 years on. J. Intern. Med., 253, 263–275.
5. Mix, E., Goertsches, R. and Zett, U.K. (2006) Immunoglobulins—basic considerations. J. Neurol., 253(Suppl 5), V9–V17.
6. Glanville, J., Zhai, W., Berka, J., Telman, D., Huerta, G., Mehta, G.R., Ni, I., Mei, L., Sundar, P.D., Day, G.M. et al. (2009) Precise determination of the diversity of a combinatorial antibody library gives insight into the human immunoglobulin repertoire. Proc. Natl. Acad. Sci. USA., 106, 20216–20221.
7. Nothelfer, K., Sansonetti, P.J. and Philimon, A. (2015) Pathogen manipulation of B cells: the best defence is a good offence. Nat. Rev. Microbiol., 13, 173–184.
8. Cho, C.Y., Lai, C.C., Lee, M.L., Hsu, C.L., Chen, C.J., Chang, L.Y., Lo, C.W., Chiang, S.F. and Wu, K.G. (2017) Clinical analysis of fever of unknown origin in children: a 10-year experience in a northern Taiwan medical center. J. Microbiol. Immunol. Infect., 50, 40–45.
9. Rowley, A.H., Baker, S.C., Orenstein, J.M. and Shulman, S.T. (2008) Searching for the cause of Kawasaki disease—cytoplasmic inclusion bodies provide new insight. Nat. Rev. Microbiol., 6, 394–401.
10. Burgner, D. and Harnden, A. (2005) Kawasaki disease: what is the epidemiology telling us about the etiology? Int. J. Infect. Dis., 9, 185–194.
11. Tsai, F.J., Lee, Y.C., Chang, J.S., Huang, L.M., Huang, F.Y., Chiu, N.C., Chen, M.R., Chi, H., Lee, Y.J., Chang, L.C. et al. (2011) Identification of novel susceptibility Loci for Kawasaki disease in a Han Chinese population by a genome-wide association study. PLoS One, 6, e16853.

**Figure 2.** Difference in the sum of frequencies of dominant IgM CDR3 clonotypes between IVIG-sensitive and IVIG-resistant patients before the treatment. (A) Comparison of sums of frequencies of dominant IgM CDR3 clonotypes (defined as those with a clonotype frequency of 0.1% or higher) were calculated in the IVIG-sensitive patients (n = 30) and IVIG-resistant KD patients (n = 10). Unpaired t-test, ***P < 0.001. (B) ROC curves are applied to assess the performance of the sum of dominant IgM CDR3 frequencies as a classifier to predict the response to IVIG. The AUC is 0.91.
12. Lee, Y.C., Kuo, H.C., Chang, J.S., Chang, L.Y., Huang, L.M., Chen, M.R., Liang, C.D., Chi, H., Huang, F.Y., Lee, M.I. et al. (2012) Two new susceptibility loci for Kawasaki disease identified through genome-wide association analysis. Nat. Genet., 44, 522–525.

13. Onouchi, Y., Ozaki, K., Burns, J.C., Shimizu, C., Terai, M., Hamada, H., Honda, T., Suzuki, H., Suenaga, T., Takeuchi, T. et al. (2012) A genome-wide association study identifies three new risk loci for Kawasaki disease. Nat. Genet., 44, 517–521.

14. Stavnezer, J. and Schrader, C.E. (2014) IgH chain class switch recombination: mechanism and regulation. J. Immunol., 193, 5370–5378.

15. Hwang, J.K., Alt, F.W. and Yeap, L.S. (2015) Related mechanisms of antibody somatic hypermutation and class switch recombination. Microbiol. Spectr., 3, MDNA3-0037-2014.

16. Ko, T.M., Kuo, H.C., Chang, J.S., Chen, S.P., Liu, Y.M., Chen, H.W., Tsai, F.J., Lee, Y.C., Chen, C.H., Wu, J.Y. et al. (2015) CXCL10/IP-10 is a biomarker and mediator for Kawasaki disease. Circ. Res., 116, 876–883.

17. Fury, W., Tremoulet, A.H., Watson, V.E., Best, B.M., Shimizu, C., Hamilton, J., Kanegaye, J.T., Wei, Y., Kao, C., Mellis, S. et al. (2010) Transcript abundance patterns in Kawasaki disease patients with intravenous immunoglobulin resistance. Hum. Mol. Genet., 19, 865–873.

18. Shimizu, C., Jain, S., Davila, S., Hibberd, M.L., Lin, K.O., Molkara, D., Frazer, J.R., Sun, S., Baker, A.L., Newburger, J.W. et al. (2011) Transforming growth factor-beta signaling pathway in patients with Kawasaki disease. Circ. Cardiovasc. Genet., 4, 16–25.

19. Lv, Y.W., Wang, J., Sun, L., Zhang, J.M., Cao, L., Ding, Y.Y., Chen, Y., Dou, J.J., Huang, J., Tang, Y.F. et al. (2013) Understanding the pathogenesis of Kawasaki disease by network and pathway analysis. Comput. Math. Methods Med., 2013, 989307.

20. Calis, J.J. and Rosenberg, B.R. (2014) Characterizing immune repertoires by high throughput sequencing: strategies and applications. Trends Immunol., 35, 581–590.

21. Georgiou, C., Ippolito, G.C., Beausang, J., Busse, C.E., Wardemann, H. and Quake, S.R. (2014) The promise and challenge of high-throughput sequencing of the antibody repertoire. Nat. Biotechnol., 32, 158–168.

22. Greiff, V., Miho, E., Menzel, U. and Reddy, S.T. (2015) Bioinformatic and statistical analysis of adaptive immune repertoires. Trends Immunol., 36, 738–749.

23. Manihot, C., Yeung, R.S., Clarizia, N.A., Chahal, N. and McCrindle, B.W. (2009) Kawasaki disease at the extremes of the age spectrum. Pediatrics, 124, e410–e415.

24. Stavnezer, J. and Amemiya, C.T. (2004) Evolution of isotype switching. Sem. Immunol., 16, 257–275.

25. McCrindle, B.W., Rowley, A.H., Newburger, J.W., Burns, J.C., Bolger, A.F., Gewitz, M., Baker, A.L., Jackson, M.A., Takahashi, M., Shah, P.B. et al. (2017) Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association. Circulation, 135, e927–e999.

26. Robinson, W.H. (2015) Sequencing the functional antibody repertoire—diagnostic and therapeutic discovery. Nat. Rev. Rheumatol., 11, 171–182.

27. Newburger, J.W., Takahashi, M., Gerber, M.A., Gewitz, M.H., Tani, L.Y., Burns, J.C., Shulman, S.T., Bolger, A.F., Ferrieri, P., Baltimore, R.S. et al. (2004) Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Pediatrics, 114, 1708–1733.

28. Kiyotani, K., Mai, T.H., Yamaguchi, R., Yew, P.Y., Kulis, M., Orgel, K., Imoto, S., Miyano, S., Burks, A.W. and Nakamura, Y. (2018) Characterization of the B-cell receptor repertoires in peanut allergic subjects undergoing oral immunotherapy. J. Hum. Genet., 63, 239–248.

29. Giudicelli, V., Chaume, D. and Lefranc, M.P. (2004) IMGT/GENE-DB: a comprehensive database for human and mouse immunoglobulin and T cell receptor genes. Nucleic Acids Res., 33, D256–D261.

30. Lefranc, M.P., Giudicelli, V., Kaas, Q., Duprat, E., Jabado-Michaloud, J., Scaviner, D., Ginestoux, C., Clement, O., Chaume, D. and Lefranc, G. (2005) IMGT, the international Immunogenetics information system. Nucleic Acids Res., 33, D593–D597.

31. Langmead, B. and Salzberg, S.L. (2012) Fast gapped-read alignment with Bowtie 2. Nat. Methods, 9, 357–359.