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# Effect of Fc- $\gamma$ Receptor Polymorphism on Rituximab-Mediated B Cell Depletion in ABO-Incompatible Adult Living Donor Liver Transplantation

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**Background.** The affinity of IgG Fc receptor (Fc $\gamma$ R) for rituximab, an anti-CD20 IgG1, differs based on single-nucleotide polymorphisms (SNPs) in Fc $\gamma$ Rs. This study aimed to explore the effect of such SNPs on clinical response to rituximab and outcomes in patients of ABO-incompatible (ABOi) living donor liver transplantation (LDLT). **Methods.** SNPs of *FCGR2A*[131H/R] and *FCGR3A*[158F/V], alleles encoding Fc $\gamma$ R, were identified in 20 patients desensitized with rituximab before ABOi LDLT. The effect of these SNPs on B cell elimination and outcomes was analyzed in the patients. **Results.** The isoform encoded by *FCGR2A* [131H/H] had a higher affinity for IgG1, and accordingly, the effects of rituximab on B cells were more profound in individuals with *FCGR2A*[131H/H] than in individuals with *FCGR2A*[131H/R or R/R]. Specifically, the time to B-cell reappearance in the peripheral blood was significantly delayed, and total serum IgM levels were significantly lower early after LDLT in individuals with *FCGR2A* [131H/H], even though these SNPs did not significantly affect the reduction of antibody group A/B antibodies. The incidence of blood stream infection was also significantly higher in individuals with *FCGR2A*[131H/H], and this SNP was associated with poor prognosis. Despite no significant effect of *FCGR3A*[158F/V] on survival after ABOi liver grafts, the incidence of infection was significantly higher in individuals with *FCGR3A*[158F/V or F/F] than in individuals with *FCGR3A*[158V/V]. **Conclusions.** Our findings indicate *FCGR* SNPs influence the effect of rituximab on B-cell depletion and are possibly predisposing factors for infectious complications after ABOi LDLT. This study will be a good foundation for further studies on larger cohorts.

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To achieve successful ABO blood group-incompatible (ABOi) kidney transplantation, the prophylactic use of rituximab, a monoclonal chimeric human-murine anti-CD20 IgG1 that depletes B cells by complement-dependent cytotoxicity, antibody (Ab)-dependent cell-mediated cytotoxicity (ADCC), and stimulation of apoptosis,<sup>1,2</sup> is currently indispensable.<sup>3</sup> Treatment with rituximab has also been employed in adult ABOi living donor liver transplantation (LDLT), and

it improved outcomes to levels close to those achieved in ABO-compatible LDLT,<sup>4</sup> although the higher incidence of severe infectious complications in ABOi LDLT cases remains to be solved. Because ADCC is initiated by interactions between the Fc segment of IgG and Fc gamma receptors (Fc $\gamma$ Rs) on monocytes, macrophages, dendritic cells, and/or natural killer (NK) cells, allelic polymorphisms on genes encoding the Fc $\gamma$ R on these immunocompetent cells potentially influence the effects

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of rituximab. One such polymorphism in the gene encoding *FCGR3A* [158F/V] (rs396991) is a single nucleotide substitution that leads to the replacement of phenylalanine (F) by valine (V) at amino acid position 158 in the IgG binding domain.<sup>5</sup> IgG1 and IgG3 bind more strongly to FcγRIIIa [158V/V] than to [158F/F], thereby increasing effector cell activity in individuals with *FCGR3A* [158V/V].<sup>6-9</sup> A single-nucleotide polymorphism (SNP) in the gene encoding *FCGR2A* [131H/R] (rs1801274) places either histidine (H) or arginine (R) at position 131.<sup>10</sup> IgG1 binds more strongly to cells that have FcγRIIa [131H/H].<sup>6,9,11</sup> These SNPs of *FCGR2A* and *FCGR3A* have been reported to be associated with the clinical outcome of rituximab in B cell non-Hodgkin lymphoma.<sup>12-14</sup> Previous basic studies have also demonstrated the correlations between *FCGR* SNPs and the effect of ADCC, which are entirely consistent with the affinity of FcγR for IgG1, showing no opposing results.<sup>15,16</sup> In contrast, some clinical analyses do not support a predictive role of *FCGR2A* and/or *FCGR3A* SNPs in the therapy of B cell malignancies with rituximab.<sup>17-19</sup> Heterogeneous features of the B-cell malignancies, such as CD20 expression levels,<sup>20</sup> HLA polymorphisms,<sup>21</sup> and a variety of intrinsic apoptotic activity,<sup>18,22</sup> or the combination of rituximab with other chemotherapy<sup>17</sup> may help explain the inconsistent objective response rate in clinical analysis. Thus, numerous reports have described the association of *FCGR2A* and *FCGR3A* SNPs with the objective response rate of B cell malignancies to rituximab. However, none of the other analyses has shown the impact of SNPs on the effect of rituximab in ABOi organ transplantation. This is the first report on the influence of SNPs of *FCGR2A* and *FCGR3A* on the normal B-cell elimination, total serum immunoglobulin levels, and outcomes rituximab-based desensitization therapy for ABOi LDLT.

## MATERIALS AND METHODS

### Study Population and Immunosuppression Regimen

The cases (n = 20) that received ABOi LDLT under rituximab-based immunosuppressive regimen from April 2007 to March 2016 at the Hiroshima University were enrolled in this study. Life-threatening, severe comorbidities were not observed preoperatively in this cohort. Rituximab (375 mg/m<sup>2</sup>) was intravenously administered 2 weeks before the transplantation, whereas tacrolimus and mycophenolate mofetil were orally administered 2 weeks before the transplantation, and the target trough levels of tacrolimus were 5 to 10 ng/mL. Plasma exchange or double filtration plasmapheresis was performed to preserve the spleen in recipient of LDLT when high titer of the Ab was reduced. Simultaneous splenectomy had not been performed for immunosuppression. After transplantation, IVIg therapy was appropriately performed, which is required to maintain the total IgG levels in sera within the normal limit, without the aim of immunosuppression. This study was approved by the Institutional Review Board of Hiroshima University (No Hi-77, M625), and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki, 1975. A written informed consent was obtained from all the patients.

### Prophylactic Antibiotic Therapy

Antimicrobial prophylaxis consisted of intravenous cefmetazole (1.0 g/instance) (CEFMETAZON for intravenous

injection; Daiichi Sankyo Company, Limited, Tokyo, Japan) administration shortly before surgery and every 6 hours during surgery; thereafter, a dosage of 2.0 g/d was maintained for 4 days.

### Analysis of SNPs of *FCGR2A* [131H/R] and *FCGR3A* [158F/V]

Determination of SNPs of *FCGR2A* [131H/R] (rs1801274) and *FCGR3A* [158F/V] (rs396991) was done by restriction-fragment length polymorphism analysis. Genomic DNA was isolated from peripheral blood mononuclear cells or liver-derived mononuclear cells using Wizard SV Genomic DNA Purification System (Promega Corporation, Madison, WI), per manufacturer's instructions. The genomic region containing the polymorphism was amplified by polymerase chain reaction, and the amplicon was purified using commercially available polymerase chain reaction product purification kit, KOD FX (TOYOBO, Fukui, Japan). Restriction-fragment length polymorphism analysis was done by enzymatic digestion with restriction enzymes, BstUI and N1aIII, (New England BioLabs, Ipswich, MA) for *FCGR2A* and *FCGR3A*, respectively. The enzymatically digested restriction fragments were separated on 2.5% agarose gels.

### Residual B-Cell Analysis in Peripheral Blood and Spleen by Flow Cytometry

We performed spleen biopsy during ABOi LDLT desensitization with rituximab in all patients, except in patients who underwent splenectomy before transplantation. Our objective was to validate the elimination of B cells from the spleen and determine if the patient required splenectomy. The cells were stained with fluorescein isothiocyanate-conjugated anti-IgM (BD Pharmingen) and PE-conjugated anti-CD19 (BD Pharmingen) and analyzed using a fluorescence-activated cell sorter Calibur flow cytometer (Becton Dickinson). The rate of residual B cells in splenocytes and peripheral blood mononuclear cells was determined by selecting cells gated on IgM<sup>+</sup> and CD19<sup>+</sup> lymphocytes.

We performed splenectomy only if B cells were clearly detected in the spleen by flow cytometry analyses of the biopsy specimens at the time of transplantation (ie, in cases where more than 1% of IgM<sup>+</sup> CD19<sup>+</sup> cells were detected among the splenocytes).

### Definition of Blood Stream Infection

Blood stream infections (BSI) accompanied by a bacteriological proof and clinical symptoms within 3 months after LDLT regardless of the presence or absence of other infectious diseases.

### Statistical Analysis

The results were statistically analyzed using the Mann-Whitney *U* test. The Pearson  $\chi^2$  test was used to analyze the incidence rate. Overall survival was calculated with the Kaplan-Meier method, and the statistical differences were determined by the log-rank test. Values with *P* less than 0.05 were considered as statistically significant.

## RESULTS

### Demographics and Characteristics of Patients

Genotyping data for *FCGR2A* [131H/R] and *FCGR3A* [158F/V] of 20 patients who received ABOi LDLT were

obtained, which revealed that 10 patients were homozygous for *FCGR2A* [131H/H], the other 10 were *FCGR2A* [131R/R or R/H], whereas 9 patients were homozygous for *FCGR3A* [158V/V], 11 were *FCGR3A* [158F/F or F/V]. Comparative analyses of the characteristics of patients with *FCGR2A* and *FCGR3A* SNPs are listed in Table 1. The incidence of severe biliary complications was significantly higher

in patients with *FCGR2A* [131H/H] than that in patients with *FCGR2A* [131R/R or R/H]. The number of *FCGR2A* [131R/R or R/H] patients treated with radiofrequency ablations for hepatocellular carcinoma (HCC) was significantly higher as compared with the *FCGR2A* [131H/H] patients. Further, the proportion of hepatitis B virus cases for *FCGR3A* [158F/F or F/V] patients was significantly lower as compared

**TABLE 1.****Characteristics of ABO-incompatible LDLT recipients with *FCGR2A* and *FCGR3A* polymorphism**

	<i>FCGR2A</i>	<i>FCGR2A</i>	<i>P</i>	<i>FCGR3A</i>	<i>FCGR3A</i>	<i>P</i>
	131HH (n = 10)	131H/R or R/R (n = 10)		158VV (n = 9)	158V/F or F/F (n = 11)	
Sex			1			0.36
M	8	8		8	8	
F	2	2		1	3	
Age	54.6 ± 6.0	48.2 ± 12.0	0.15	49.0 ± 11.9	53.4 ± 7.7	0.34
History of abdominal surgery	2	2	1	1	3	0.37
MELD	16.4 ± 5.0	14.7 ± 4.3	0.42	16.0 ± 5.5	15.2 ± 4.0	0.7
Donor age	37.7 ± 10.4	32.9 ± 13.2	0.38	34.7 ± 14.4	35.8 ± 10.0	0.83
Liver disease						
HCV	6	4	0.37	3	7	0.18
HBV	1	2	0.53	3	0	0.038
Alcohol	1	2	0.53	2	1	0.41
NASH	2	0	0.14	0	2	0.18
Budd-Chiari	0	1	0.3	1	0	0.26
PBC	0	1	0.3	0	1	0.35
HCC	6	6	1	7	5	0.14
TACE	2	5	0.08	4	3	0.92
RFA	0	3	0.045	1	2	0.51
Preoperative plasma exchange (course)	2.6 ± 1.6	3.1 ± 1.5	0.48	2.7 ± 1.2	3.0 ± 1.8	0.64
GRWR	0.90 ± 0.25	0.82 ± 0.13	0.4	0.79 ± 0.13	0.93 ± 0.22	0.11
Cold ischemic time, min	100 ± 25.0	104 ± 30.2	0.76	109 ± 28.1	97 ± 26.3	0.35
Blood loss, mL	4761 ± 2461	3977 ± 2264	0.46	5161 ± 2136	3720 ± 2387	0.18
Operation time, min	758 ± 82.2	767 ± 77.8	0.79	786 ± 60.4	743 ± 88.0	0.23
Splenectomy			0.65			0.34
Yes	4	5		3	6	
No	6	5		6	5	
Surgical complications						
Postoperative bleeding	2	1	0.53	0	3	0.09
Dindo-Clavien						
IIIa	1	0		0	1	
IIIb	1	1		0	2	
IV	0	0		0	0	
V	0	0		0	0	
Intraperitoneal abscess	1	0	0.3	0	1	0.35
Dindo-Clavien						
IIIa	1	0		0	1	
IIIb	0	0		0	0	
IV	0	0		0	0	
V	0	0		0	0	
Biliary stenosis, cholangitis	7	3	0.07	5	5	1
Dindo-Clavien						
IIIa	3	2		3	2	
IIIb	0	1		1	0	
IV	0	0		0	0	
V	4	0	0.025	1	3	0.36

Data are shown as mean ± SD or the number of cases.

M, male; F, female; MELD, model for end-stage living disease score; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; GRWR, graft/recipient weight ratio; HCV, hepatitis C virus; HBV, hepatitis B virus.

with that for the *FCGR3A* [158V/V] patients. However, these diseases did not seem to influence patient mortality. No other statistically significant differences in patient characteristics were observed. Neither elevation of antibody group isoagglutinin titers nor Ab-mediated rejection was observed after transplantation in any of the patients, as previously reported.<sup>23</sup>

#### No Differences Were Observed in the Proportion of Residual B cells in the Spleen Regardless of SNPs of *FCGR2A* and *FCGR3A*

The immunocompetent cells with *FcγRIIa* H-allele have been reported to have a remarkably higher affinity to human IgG1 than those with R-allele, raising the question of whether there is difference in effects of rituximab with respect to the *FCGR2A* SNPs.<sup>6,9,11</sup> Similarly, in *FcγRIIIa*, immunocompetent cells with V-allele has a slightly higher affinity for IgG1 than those with F-allele,<sup>6-9</sup> although the affinity of *FcγRIIIa* to IgG1, per se, is generally lower than that of *FcγRIIa*, regardless of genotypes.<sup>9</sup> Despite such theoretical projection, flow cytometry analyses for biopsy specimens of the spleen collected at the time of transplantation revealed no differences in the proportion of residual B cells regardless of the SNPs of *FCGR2A* and *FCGR3A* (Figure S1, SDC, <http://links.lww.com/TXD/A42>).

#### Effects of Rituximab on B Cells Were More Profound in Individuals With *FCGR2A* [131H/H] Than in Individuals With *FCGR2A* [131H/R or R/R]

Regardless of the *FcγR* genotypes, B cells in the peripheral blood were unexceptionally abrogated at 1 week after the administration of a single dose of rituximab, and remained undetectable for 3 months after LT (Figure 1A). However, the time of B-cell reappearance in the peripheral blood was significantly delayed in individuals with *FCGR2A* [131H/H], consistent with a higher affinity of the isoform encoded by *FCGR2A* [131H/H] to IgG1 (Figure 1A). In addition, the total IgM levels in serum were significantly lower (beyond the lower limit of standard value) in the early period after LT in individuals with *FCGR2A* [131H/H] than in *FCGR2A* [131H/R or R/R] recipients (Figure 1B). Such a trend in serum IgM levels was not observed for the *FCGR3A* SNPs, probably reflecting the lower affinity of *FcγRIIIa* to IgG1 than that of *FcγRIIa*. Different from serum IgM levels, serum IgG levels were influenced to a similar level by administering IVIg, which was generally indicated in patients with hypoglobulinemia. Hence, we analyzed both the serum IgG levels and total administered dose of IVIg in recipients of LT. The IgG levels were kept around lower limit of standard value by administering IVIg during the early period after LT, with no difference with respect to SNPs of both *FCGR2A* and *FCGR3A* (Figure 1C). Noticeably, a significantly larger amount of IVIg was required in individuals with *FCGR2A* [131H/H] to maintain serum IgG levels comparable to those in individuals with *FCGR2A* [131H/R or R/R] (Figure 1D). This result suggests that net production of IgG might be further suppressed and/or existing IgG might be further consumed for immune defense against pathogens in individuals with *FCGR2A* [131H/H].

#### *FCGR* SNPs Influence the Incidence of Infection and Outcomes of ABOi LDLT

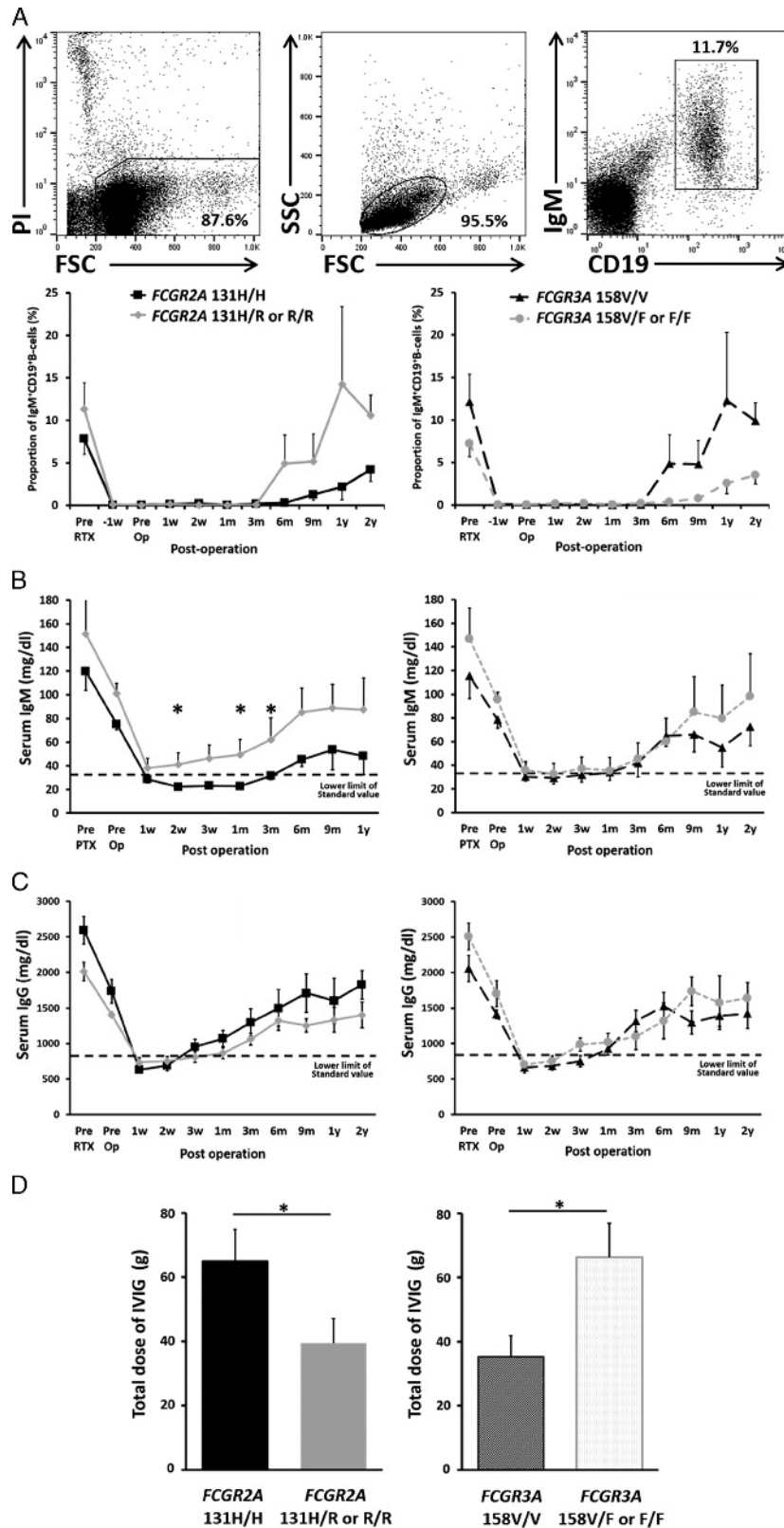
The severely suppressed immunoglobulin production in individuals with *FCGR2A* [131H/H] raised a question as to

whether they are susceptible to infectious complications. Consistent with this assumption, the incidence of BSI was significantly higher in individuals with *FCGR2A* [131H/H] than that in individuals with *FCGR2A* [131H/R or R/R] (Figure 2A), and the prognosis of individuals with *FCGR2A* [131H/H] was markedly poor, whereas preferable outcomes were seen in individuals with *FCGR2A* [131H/R or R/R] (Figure 2B). With respect to *FCGR3A* SNPs, although the impact of the polymorphism on the prognosis was not remarkable (Figure 2B), significantly higher incidence of BSI was observed in individuals with *FCGR3A* [158F/F or F/V] than in individuals with *FCGR3A* [158V/V] (Figure 2A). We have recently demonstrated that the *FCGR3A* SNP in recipients of LT was greatly associated with the susceptibility to severe posttransplant infections, ie, a significantly higher incidence of BSI was observed in the individuals with *FCGR3A* [158F/V or F/F] than that in the individuals with *FCGR3A* [158V/V].<sup>24</sup> This finding might reflect a fact that the affinity for IgG3, which induces vigorous complement activation and ADCC, is remarkably higher in *FcγRIIIa* than that in *FcγRIIa*, and *FCGR3A* [158F/F or F/V] has much less affinity than *FCGR3A* [158V/V].<sup>9,24</sup> Hence, *FCGR3A* SNPs likely influence innate components of humoral immunity.

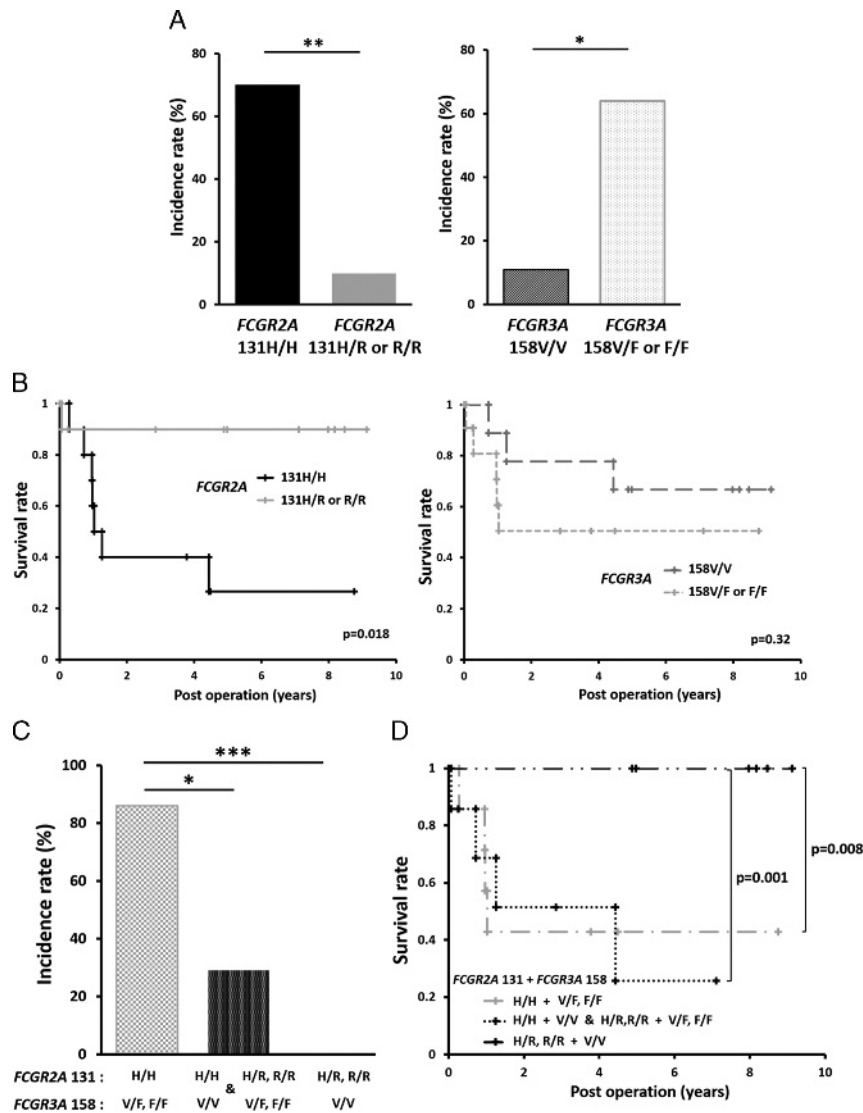
As *FcγR* classes differ in cell distribution and affinity for IgG subclasses, the combination of *FCGR2A* and *FCGR3A* SNPs possibly further stratifies the incidence of infectious complications. The low-risk combination, which consists of *FCGR2A* [131R/R or R/H] and *FCGR3A* [158V/V], showed excellent outcome: 8-year survival rate of 100%, whereas the other combinations (*FCGR2A* [131H/H] and *FCGR3A* [158F/F or F/V or V/V] or *FCGR2A* [131R/R or R/H] and *FCGR3A* [158F/F or F/V]) had significantly poor prognosis, indicating that SNPs of *FCGR2A* and *FCGR3A* influence the outcome of ABOi LDLT desensitized by rituximab (Figures 2C and D). Regarding cause of the death, 7 of the 8 death patients in the present study died of sepsis. These patients frequently sustained other complications, such as cholangitis, pneumonia, recurrence of hepatitis C virus, or acute rejection. All 7 patients suffered from BSI post-LT. Even though these patients successfully recovered from early phase BSI, they eventually died of sepsis. Only 1 patient died 5 years after LT because of HCC recurrence. These results suggest that a strong association exists between survival and infectious complications.

## DISCUSSION

B-cell desensitization using rituximab has become an established strategy for ABOi LDLT. However, the higher incidence of severe infectious complications in ABOi LDLT cases than that in ABO-compatible LDLT cases remains to be resolved. The elimination of B cells sometimes caused hypoglobulinemia for both of IgG and IgM. A critical role of natural IgM in the immediate defense against several antigens has been well demonstrated,<sup>25</sup> and previous studies suggested that polyvalent IgM-enriched human IVIg preparations are beneficial for obtaining favorable outcomes in sepsis.<sup>26</sup> Because the IgM-enriched IVIg preparation is not available in Japan, polyclonal IgG could be solely supplemented with IVIg administration in the present treatment regimen. Hence, total IgM levels in sera are not influenced by administering IVIg; in other words, the total IgM levels



**FIGURE 1.** Effects of rituximab on B cells were more profound in individuals with FCGR2A [131H/R or R/R]. A, A single dose of rituximab (375 mg/m<sup>2</sup>) was administered 2 weeks before transplantation. The proportion of IgM<sup>+</sup>CD19<sup>+</sup> B cells in peripheral blood mono nuclear cells were determined by flow cytometry at different time points. Each point represents the mean and standard error of the mean at the individual time point as described. Representative gating strategy for the flow cytometry were shown. B, C, Total IgM (B) and IgG (C) levels in serum were analyzed at different time points as described. Dotted lines indicate lower limits of standard value. Each point represents the mean and standard error of the mean at the individual time point. D, Total dose of IVIG administered within 30 days after operation were shown. Data represent mean and standard error of the mean. The results were statistically analyzed using the Mann-Whitney *U* test. (FCGR2A [131H/H]: n = 10/[131R/R or R/H]: n = 10, FCGR3A [158V/V]: n = 9/[158F/F or F/V]: n = 11, \**P* < 0.05). RTX, rituximab.



**FIGURE 2.** FCGR SNPs influence the incidence of infection and outcomes of ABOi LDLT. A, The incidence rate of BSIs within 3 months were shown. B, Postoperative survival rate were shown with the Kaplan-Meier method. (C) The incidence rate of BSIs within 3 months by the combination of *FCGR2A* and *FCGR3A* SNPs were shown. *FCGR2A* [131R/R or R/H] and *FCGR3A* [158V/V] is low risk combination ( $n = 6$ ). *FCGR2A* [131H/H] and *FCGR3A* [158F/F or F/V] is high risk combination ( $n = 7$ ). *FCGR2A* [131H/H] and *FCGR3A* [158V/V] or *FCGR2A* [131R/R or R/H] and *FCGR3A* [158F/F or F/V] are both high risk and low risk combinations ( $n = 7$ ). \* $P < 0.05$ , \*\*\* $P < 0.001$ . D, Postoperative survival rate by the combination of *FCGR2A* and *FCGR3A* SNPs were shown with the Kaplan-Meier method. The Pearson  $\chi^2$  test was used to analyze the incidence rate, and the statistical differences of overall survival were determined by the log-rank test. Values with  $P < 0.05$  were considered as statistically significant.

might directly reflect the few B cells remaining even after administration of rituximab. In this study, by evaluating the total IgM levels, we analyzed the effect of rituximab on the depletion of normal B cells with low variation in CD20 expression. This approach may have less bias than analyzing the clinical objective response rate of B cell malignancies with phenotypic variations to a therapy.<sup>18,20-22</sup> Our results for binding affinities to IgG1 were consistent for *FCGR2A* SNP, but not for *FCGR3A* SNP. Since ADCC mediated through Fc $\gamma$ R, carried by immunocompetent cells such as macrophages and NK cells, plays a critical role in the effect of IgG1 Ab therapy,<sup>27</sup> Fc $\gamma$ RIIa having higher affinity for IgG1 may have a stronger association with the effect of rituximab than with the lower affinity of Fc $\gamma$ RIIIa for IgG1. Another reason for the remarkable association of the effect of rituximab with *FCGR2A* SNP but not with *FCGR3A* SNP

might be the difference in cell distribution of Fc $\gamma$ R classes, that is, Fc $\gamma$ RIIa is expressed on neutrophils and macrophages, whereas Fc $\gamma$ RIIIa is expressed on NK cells and macrophages.<sup>14</sup> Considering the fact that the vast majority of immunocompetent cells bearing Fc $\gamma$ RIIIa are NK cells<sup>15</sup> and our previous finding that cytotoxic activity of NK cells is inhibited in patients suffering from severe liver failure who require liver transplantation,<sup>28</sup> it is likely that ADCC-mediated B cell elimination through Fc $\gamma$ R caused by rituximab administered before LDLT is dependent on the cytotoxicity of neutrophils and macrophages bearing Fc $\gamma$ RIIa more than that of NK cells bearing Fc $\gamma$ RIIIa.

Although B cells in the spleen at the time of transplantation were abrogated regardless of *FCGR2A* and *FCGR3A* SNPs, the time of B cell reappearance in the peripheral blood was significantly delayed and the total IgM levels in serum were

significantly lower at the early period after LT in individuals with *FCGR2A* [131H/H]. This finding is consistent with the higher affinity of the isoform encoded by *FCGR2A* [131H/H] to IgG1 than that of the isoform encoded by *FCGR2A* [131H/R or R/R]. Taking into account a recent basic study using a mouse model, which demonstrated that all B cells in the spleen were depleted while those in the bone marrow remained detectable at the early period after injection of anti-CD20 monoclonal Ab,<sup>29</sup> it is possible that the *FCGR2A* SNP is associated with the levels of remnant B cells in the bone marrow of LT patients treated with rituximab, thereby influencing the pace of B cell recovery in peripheral blood.

Immunoglobulin-directed activity plays a pivotal role in the innate immunity against microbes. Immunoglobulins with appropriate constant region isotypes (IgG1 and IgG3) can induce complement-dependent activities. In addition, they can recruit immunocytes and induce various activities through the interaction between IgG and Fc $\gamma$ R on innate immune cells. This includes stimulation of bacterial phagocytosis by macrophages, induction of ADCC by macrophages or NK cells, and the generation of an oxidative burst from neutrophils. Further, IgM can also induce complement-dependent activities. Hence, the levels of IgG and/or IgM might play a key role in postoperative infectious complications. Since the isoform encoded by *FCGR3A* [158V] has greater affinity to both IgG1 and IgG3 than those encoded by *FCGR3A* [158F],<sup>9</sup> the *FCGR3A* genotype may influence the susceptibility to infections in patients treated with immunosuppressive drugs, which reduce adaptive immunity but not innate immunity. In the present study, IVIg therapy, which is required to maintain the total IgG levels in sera within the normal limit, was appropriately performed after transplantation. Susceptibility to infectious complications may, thus, be determined by a complex interplay between the aforementioned factors. Here, we have proposed that an association between Fc $\gamma$ R IIa polymorphism and the effect of rituximab on B cell depletion leads to a significant difference in IgM levels in sera. Since a previous report has demonstrated a critical role of natural IgM in the immediate defense against several antigens,<sup>25</sup> it is likely that IgM levels partially influence the incidence of infection after transplantation. In the present study, we have observed a trend such that patients with BSI have lower IgM levels in comparison to those without BSI, although this difference in IgM levels was not found to be statistically significant. Thus, the reduction in the IgM levels in sera observed upon B cell depletion by rituximab may be attributed to the susceptibility to severe infections in association with *FCGR2A* polymorphism.

As we reported previously, the affinity of Fc $\gamma$ RIIIa for IgG3, an important aspect of innate immune defense against microbes, is critical under immunosuppressive conditions after LDLT.<sup>24</sup> Since the affinity of Fc $\gamma$ RIIIa for IgG3 is much higher than that of Fc $\gamma$ RIIa,<sup>9</sup> *FCGR3A* SNP is likely to have a stronger association with innate immune defense. The combination of polymorphisms in *FCGR2A* influencing the effect of rituximab and *FCGR3A* influencing the innate defense activity might be a predisposing factor for infectious complications and could predict mortality after ABOi LT. This study provides a foundation for further prospective studies on a larger scale.

In conclusion, we demonstrated that the effects of rituximab on B cells were more profound in *FCGR2A* [131H/H]

than in *FCGR2A* [131H/R or R/R] recipients of ABOi LDLT. The higher incidence of infectious complications in *FCGR2A* [131H/H] recipients and their poor prognosis could potentially be attributed to this polymorphism. The combination of *FCGR2A* and *FCGR3A* SNPs further stratifies the incidence of infectious complications, ie, *FCGR2A* [131H/H] and *FCGR3A* [158F/F or F/V] displayed the highest incidence of infectious complications after ABOi LDLT, suggesting that strict management might be required to improve outcomes. Thus, SNPs of *FCGR2A* and *FCGR3A* are likely to be predisposing factors for severe infectious complications after ABOi LDLT, despite the lack of a significant influence of these SNPs on the reduction of antibody group A/B antibodies. This study can be treated as a good foundation, based on which, further analyses with larger cohorts can be carried out.

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