Two Immunoglobulin G Fragment C Receptor Polymorphisms Independently Predict Response to Rituximab in Patients With Follicular Lymphoma

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Purpose: **Although rituximab is now routinely used in the treatment of B-cell non-Hodgkin's lymphoma, the mechanism of its antitumor effect is not clear. One potential mechanism of action involves antibody-dependent cellular cytotoxicity (ADCC). Two aspects of ADCC influence the effectiveness of this process: the susceptibility of tumor cells and the activation of effector cells via their immunoglobulin G fragment C receptors (FcγRs). Several FcγR polymorphisms have been identified that may affect the killing function of natural killer cells and macrophages.**

Patients and Methods: **The pretreatment tumor cells from 43 patients with follicular lymphoma were tested for their intrinsic susceptibility to rituximab-mediated ADCC. In addition, the FcγRIIIa (CD16) and FcγRIIa (CD32) polymorphisms were determined in an expanded group of 87 patients. The results were then correlated with clinical outcome of these patients.**

Results: **No difference was found between the susceptibility of tumors from patients who clinically responded to rituximab versus those who did not respond. Conversely, both the FcγRIIIa 158 valine/valine and the FcγRIIa 131 histidine/histidine genotypes were found to be independently associated with the response rate and freedom from progression.**

Conclusion: **These data support the hypothesis that ADCC plays an important role in the clinical effect of rituximab at the level of the effector cell. It will be important to include information on Fc receptor polymorphisms in future trials of rituximab therapy.**

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THE CHIMERIC anti-CD20 monoclonal antibody (mAb), rituximab, is an effective treatment for low-grade B-cell non-Hodgkin's lymphoma. However, the exact mechanism of its antitumor effect is not clear. A possible role for antibody-mediated apoptosis has been suggested by studies with rituximab in vitro on lymphoma cell lines or on chronic lymphocytic leukemia cells.[1-3] Immune mechanisms, including antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity, may also be involved.[4,5] This notion has been supported by several observations: rituximab had significantly reduced antitumor effect in fragment C receptor (FcγR)–deficient mice.[6] Lymphoma cell lines and freshly isolated follicular lymphoma cells are susceptible to ADCC and to complement-mediated cytotoxicity in the presence of rituximab.[4,5,7,8] Lymphoma cells of various histologies showed a pattern of sensitivity to in vitro complement-mediated cytotoxicity that is consistent with their clinical sensitivity to rituximab treatment.[8] The therapeutic effect of rituximab against a human CD20-transfected murine T lymphoma cell line was abolished in complement (C1q)-deficient mice.[9] However, in our recent study, the susceptibility of tumor cells to complement-mediated cytotoxicity in vitro did not correlate with the clinical outcome to rituximab therapy.[7]

In ADCC, the antibody binds to tumor cells and then is engaged by effector cells via their receptors for immunoglobulin G (FcγRs).[10] Recently, a role for FcγR has been suggested by the report that a polymorphism of FcγRIIIa was associated with tumor response in follicular lymphoma patients treated with rituximab as first-line therapy.[11] Patients with homozygous 158 valine/valine (V/V) alleles of FcγRIIIa showed a higher response rate to rituximab treatment. It is known that the FcγRIIIa of V allele has a higher affinity to human IgG1 than does the phenylalanine (F) allele and that cells bearing the FcγRIIIa V allele mediate ADCC more effectively.[12,13]

In this study, we performed in vitro ADCC assays on pretreatment tumor cells from patients subsequently treated with rituximab. In addition, we determined the polymorphisms of two activating Fc receptors. We found that the FcγR polymorphisms each, independently, correlated with tumor response to rituximab.

PATIENTS AND METHODS

Patient Population

This study included 87 patients with follicular lymphoma, who were treated with rituximab at Stanford Medical Center between 1993 and 2003. They were selected because of the availability of their lymphoma tumor cells, peripheral blood or serum samples, and their known clinical response to rituximab. The pathology of all patient cases was reviewed. There were 47 patients with follicular small cleaved, 35 patients with follicular mixed, and five patients with follicular large-cell lymphoma. Fifteen patients had received rituximab as their first-line therapy. Seventy-two patients had

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received chemotherapy before rituximab, including 10 patients who had prior bone marrow transplantation. No patients received chemotherapy within the 2 months before rituximab treatment. Eighty-one patients had four weekly infusions of rituximab at 375 mg/m^2, five patients had eight weekly infusions of 375 mg/m^2, and one patient had four weekly infusions of 250 mg/m^2. Clinical responses were determined by physical examination and computed tomography scans between 1 and 3 months after last rituximab infusion and every 3 months thereafter. These responses were scored according to the Cheson criteria.[14] Maximal clinical responses were observed at 1 to 3 months in all but three patients, who had partial responses at 1 to 3 months and showed additional tumor shrinkage at later time points. Pretreatment tumor cells were available in 43 patients and were used for in vitro ADCC assay. FcγR polymorphisms were analyzed in all 87 patients. This study was conducted according to a protocol approved by the institutional review board of our institution, and informed consent was obtained from all patients for the use of tissue samples and the analysis of clinical information.

Tumor Cells

Suspensions of pretreatment tumor cells isolated from lymph nodes were cryopreserved in liquid nitrogen. For ADCC assay, the tumor cells were thawed and subjected to Ficoll-Paque PLUS (Amersham Pharmacia Biotech, Piscataway, NJ) gradient centrifugation to remove dead cells. The viability of tumor cells, determined by trypan blue dye exclusion at the time of assay, always exceeded 90%. The percentage of tumor cells in each sample was estimated by staining with antibodies specific for kappa or lambda light chains.

ADCC Assay

Lymphoma cells were labeled with chromium-51 (^{51}Cr) by incubating 3×10^6 cells with 450 μCi of ^{51}Cr (Amersham Pharmacia Biotech) for 2 hours at 37°C. Cells were washed with RPMI-1640, and then incubated for 30 minutes at 37°C with antibodies (at 10 μg/mL). Excess antibodies were removed by washing with medium. Mononuclear cells were obtained by Ficoll-Hypaque centrifugation of peripheral blood of a healthy donor (with FcγRIIIa 158 V/V genotype) and used as effector cells. One $\times 10^4$ ^{51}Cr-labeled target cells were incubated for 4 hours at 37°C with the indicated number of effector cells in 200 μL of RPMI-5 medium (RPMI-1640, 10 mmol/L HEPES, 5% heat-inactivated human AB serum, 1% L-glutamine). Fifty microliters of medium was collected after 4 hours of incubation and counted in a MicroBeta 1450 scintillation counter (Wallac, Turku, Finland). Spontaneous ^{51}Cr release was determined in the absence of effector cells. Maximal ^{51}Cr release was determined by lysis with 0.5% Triton X-100. All samples were assayed in triplicate. The specific ^{51}Cr release was determined by subtracting the spontaneous ^{51}Cr release from that of the treatment wells, then dividing the result by the maximal ^{51}Cr release minus spontaneous ^{51}Cr release. All the tumor samples had coexistent T cells of variable degree (Table 1). To compare different tumor samples, the specific ADCC is calculated by dividing the specific ^{51}Cr release in rituximab-treated samples minus ^{51}Cr release in control IgG1-treated samples by the percentage of CD20-positive cells in individual samples.

Analysis of FcγRIIIa and FcγRIIa Polymorphisms

Genomic DNA was prepared from tumor cells or from peripheral-blood mononuclear cells using a DNA extraction kit (Qiagen, Valencia, CA). In six patients, DNA was prepared from the serum using a described method.[15] Genotyping of FcγRIIIa 158 V/F and FcγRIIa 131 histidine (H)/arginine (R) polymorphism was performed by a polymerase chain reaction followed by allele-specific restriction enzyme digestion.[16,17] All genotyping of FcγRIIIa polymorphism was confirmed by direct sequencing of the region of interest.

Statistical Analysis

Differences in the means of ADCC killing were tested by single-factor analysis of variance test and checked by the Kruskal-Wallis (nonparametric) test. The clinical responses of the patients were compared using a two-tailed Fisher's exact test (PRISM for Macintosh; GraphPad Software, San Diego, CA). A logistic regression analysis including age (≥ or < 60 years), stage (III *v* IV), presence of bulky disease, number of extranodal sites (≥ two or < two), prior bone marrow transplantation, and FcγRIIa and FcγRIIIa genotype was used to identify independent prognostic variables influencing the clinical responses (StatView 5.0.1; SAS Inc, Cary, NC).

Table 1. Patient Characteristics According to Response to Rituximab Treatment

Characteristic	No Response (n = 15)	Partial Response (n = 16)	Complete Response (n = 12)	All Patients (N = 43)
Sex				
Male	11	9	7	27
Female	4	7	5	16
Age, years				
Mean	49	56	52	52
SD	8.8	14.9	7.5	11.5
Pathology				
FSC	10	6	6	22
FM	5	8	5	18
FLC	0	2	1	3
Number of prior chemotherapy treatments				
Range	1-3	0-6	0-5	0-6
Median	2.0	2.0	1.0	2.0
Prior transplantation therapy	2	3	2	7
Bulky disease	10	5	5	20
Stage				
III	2	4	4	10
IV	13	12	8	33
≥ 2 extranodal sites of disease	3	3	2	8
Time between diagnosis and treatment, months				
Mean	56	69	65	63
SD	35	40	42	38
Estimated tumor cells* in biopsied samples, %				
Range	76-98	70-98	72-98	
Median	95	92	80	

Abbreviations: SD, standard deviation; FSC, follicular small cleaved; FM, follicular mixed; FLC, follicular large cell.

*Calculation described in Methods.

RESULTS

Rituximab-Mediated ADCC in Follicular Lymphoma Cells

We determined the ability of rituximab to mediate ADCC in follicular lymphoma cells. Pretreatment lymphoma cells from 43 patients were tested using effector cells isolated from one healthy donor. Rituximab-mediated ADCC was detected in all 43 patient samples (range, 13.5% to 100%). As expected, the parental murine antibody of rituximab, 2B8, which contains a mouse γ1 Fc portion and binds lymphoma cells identically to rituximab, did not mediate ADCC (data not shown).

We next attempted to relate the ADCC susceptibility of lymphoma cells from individual patients to their clinical response to rituximab therapy. We subdivided patients into nonresponders (NR), partial responders (PR), and complete responders (CR) according to their response to rituximab at the first evaluation at 1 to 3 months (Table 1). The range of ADCC varied widely in all three groups (NR, 16.9% to 80.6%; PR, 13.5% to 57.0%; CR, 20.9% to 100.0%; Fig 1). However, there was no

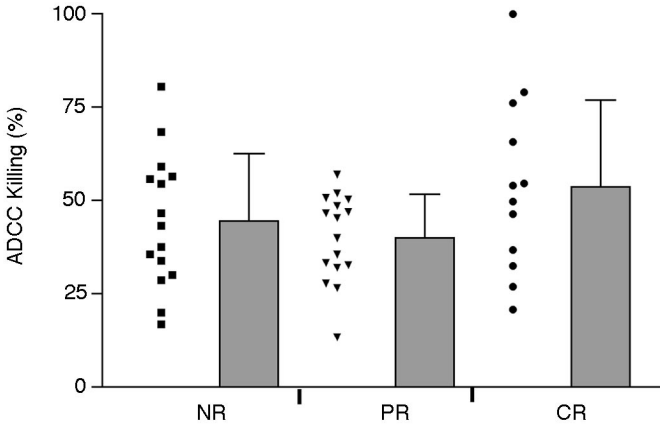

Fig 1. Rituximab-induced antibody-dependent cellular cytotoxicity (ADCC). The scatter plot in the left column of each group represents the degree of rituximab-induced ADCC (effector/target ratio at 30:1) of individual tumors. The bars represent the mean and standard deviations in each group. NR, nonresponder; PR, partial responder; CR, complete responder or complete response unconfirmed.

difference of rituximab-mediated ADCC between the three groups (means ± standard deviations: NR, 44.6% ± 18%; PR, 40.0% ± 12%; CR, 53.6% ± 23%). Additional analysis showed no relationship between rituximab-mediated ADCC and response when clinical response was scored at 6, 9, or 12 months after treatment, nor did the susceptibility to ADCC correlate with the duration of remission (data not shown). In a subgroup of 29 patients whose tumors were studied in our recent report on complement-mediated cytotoxicity,[7] the expression of CD20 on their lymphoma cells had previously been determined by flow cytometric staining. Within this subgroup, there was no correlation between the expression of CD20 and rituximab-mediated ADCC ($r = -0.03$; $P = .88$).

Clinical Response to Rituximab Therapy and FcγRIIIa 158 V/F Polymorphism

The FcγRIIIa (CD16) of V allele demonstrates higher affinity to IgG1 than the F allele and mediates ADCC more effectively. Recently, Cartron et al[11] have shown an association between FcγRIIIa 158 V/V genotype and higher response rate in patients treated with first-line rituximab. We tested for this association in our patient group, the majority of whom were treated for relapsed disease. We expanded our study group to 87 by acquiring peripheral blood or serum samples from additional rituximab-treated patients.

In this sample set, 13 patients (15%) had homozygous V/V (158 V/V), 40 (46%) had heterozygous V/F (158 V/F), and 34 (39%) had homozygous F/F (158 F/F). The three groups were not different in terms of average age at the time of treatment, number of prior chemotherapy courses, or time between diagnosis and treatment (Table 2). The response rate in patients with 158 V/F and in patients with 158 F/F was similar at all four time points (Table 3). For that reason, we grouped 158 V/F and 158 F/F together as the F carrier for statistical analysis. A significant difference was detected between the response rates of 158 V/V and F carriers (Table 3). The progression-free survival (PFS) at 2 years was 45% for patients with 158 V/V, 12% for 158 V/F, 16% for 158 F/F, and 14% for F carriers, using the

Table 2. Patient Characteristics According to FcγR Polymorphism

Characteristic	FcγRIIIa Polymorphism			All Patients	FcγRIIa Polymorphism		
	V/V (n = 13)	V/F (n = 40)	F/F (n = 34)	(N = 87)	H/H (n = 20)	H/R (n = 43)	R/R (n = 24)
Sex							
Male	9	23	16	48	10	27	11
Female	4	17	18	39	10	16	13
Age, years							
Mean	56	52	49	51	50	53	49
SD	14.5	13.0	9.1	12.0	10.4	12.4	12.1
Pathology							
FSC	9	20	18	47	10	23	14
FM	2	18	15	35	8	17	10
FLC	2	2	1	5	2	3	0
Number of prior chemotherapy treatments							
Range	0-5	0-4	0-6	0-6	0-5	0-6	0-6
Median	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Prior transplantation therapy	1	4	5	10	1	8	1
Bulky disease	6	22	18	46	9	21	15
Stage							
III	1	9	6	16	5	9	2
IV	12	31	28	61	15	34	22
≥ 2 extranodal sites of disease	3	7	2	12	5	6	1
Time between diagnosis and treatment, months							
Mean	57	68	55	61	70	54	67
SD	34	51	38	44	41	47	40

NOTE. Total No. of patients in the expanded group is 87.

Abbreviations: FcγR, immunoglobulin G fragment C receptor; FcγRIIIa, FcγR IIIa; FcγRIIa, FcγR IIa; V, valine allele; F, phenylalanine allele; H, histidine allele; R, arginine allele; SD, standard deviation; FSC, follicular small cleaved; FM, follicular mixed; FLC, follicular large cell.

Table 3. Clinical Response to Rituximab Therapy According to FcγRIIIa Polymorphism

	V/V			V/F			F/F			F Carrier* (V/F and F/F combined)			
	No. of Patients With Response	No. of Patients Assessable	%	No. of Patients With Response	No. of Patients Assessable	%	No. of Patients With Response	No. of Patients Assessable	%	No. of Patients With Response	No. of Patients Assessable	%	P†
1-3 months, objective response‡	12	13	92	21	40	53	23	34	68	44	74	59	.027
6 months, objective response	11	13	85	15	38	39	15	29	52	30	67	45	.013
9 months, objective response	9	12	75	12	36	33	11	28	39	23	64	36	.023
12 months, objective response	9	12	75	8	35	23	8	27	30	16	62	26	.002

Abbreviations: FcγRIIIA, immunoglobulin G fragment C receptor IIIA; V, valine allele; F, phenylalanine allele.

*Combination of V/F and F/F genotypes.

†Two-sided Fisher's exact test, comparing V/V with F carrier.

‡Includes partial response and complete response or complete response unconfirmed.

Kaplan-Meier estimation, with median time to progression (TTP) of 534, 148, 250, and 170 days for each group, respectively. The PFS estimate of patients with 158 V/V was significantly longer than that for patients with 158 V/F, 158 F/F, or F carriers (Fig 2).

Clinical Response to Rituximab Therapy and FcγRIIa 131 H/R Polymorphism

The FcγRIIa (CD32) is another activating FcγR that is expressed only on macrophages but not on natural killer (NK) cells. An H/R polymorphism at position 131 of FcγRIIa has been found to affect its affinity to human IgG.[17] Of our 87 patients, 20 (23%) had homozygous H/H (131 H/H), 43 (49%) had heterozygous H/R (131 H/R), and 24 (28%) had homozygous R/R (131 R/R). Once again, the three groups were not different in terms of average age at the time of treatment, number of prior chemotherapy treatments, or time between diagnosis and treatment (Table 2). Although there was no difference in the response rate at 1 to 3 months between the three groups, patients with 131 H/H showed a significantly higher response rate than the other two groups combined (H/R and R/R [R carrier]) at 6, 9, and 12 months (Table 4). This higher response rate also translated to longer remission: the PFS at 2 years was 37% for patients with 131 H/H, 13% for 131 H/R, 19% for 131 R/R, and 14% for R carrier using the Kaplan-Meier estimation with TTP of 445, 162, 158, and 158 days for each group, respectively. The PFS estimate for patients with 131 H/H was significantly longer than for patients with other genotypes (Fig 3).

We examined the possibility of an association between FcγRIIIa and FcγRIIa genotypes that might explain the correlation of the two with response rate. As shown in Table 5, there was no significant difference in the fraction of 158 V/V or F carrier in three 131 H/R genotypes. We then analyzed the combination of FcγRIIIa 158 V/V and/or FcγRIIa 131 H/H, and their relationship to rituximab response. As shown in Table 6, patients with 158 V/V and/or 131 H/H (total of 30 patients) had a significantly higher response rate than patients without either genotype at all four time points (83% *v* 54%, $P = .009$ at 1 to 3 months; 80% *v* 34%, $P = .0001$ at 6 months; 69% *v* 26%, $P = .0003$ at 9 months; 59% *v* 18%, $P = .0004$ at 12 months). The PFS estimate of patients with 158 V/V and/or 131 H/H was also significantly longer ($P = .001$), with TTP of 445 and 140 days for the two groups, respectively (Fig 4). By logistic regression analysis, FcγRIIIa 158 V/V genotype emerged as the only predictive factor for response at 1 to 3 months, whereas both the FcγRIIIa 158 V/V genotype and FcγRIIa 131 H/H genotype were identified as independent predictive factors for response at 6, 9, and 12 months (Table 7).

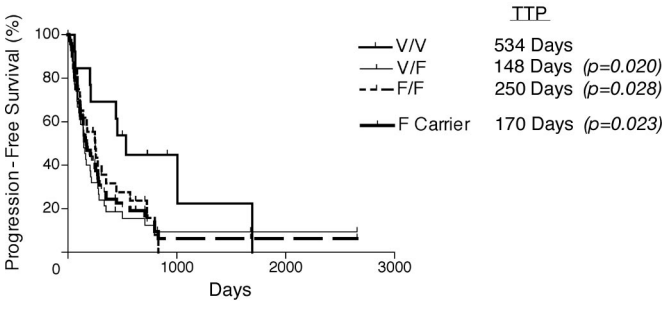

Fig 2. Kaplan-Meier estimates of progression-free survival by immunoglobulin G fragment C receptor IIIa (FcγRIIIa) 158 valine (V)/phenylalanine (F) polymorphism. Progression-free survival curves were plotted by FcγRIIIa 158 V/F genotype on all 87 patients. F carriers represent patients with either 158 V/F or 158 F/F genotype. TTP, median time to progression.

DISCUSSION

Rituximab has been integrated into routine clinical practice in the treatment of B-cell lymphoma, although the response is variable among different lymphoma types and among different patients within each type. Whether this heterogeneity is due to the difference in intrinsic properties of the tumor cells (eg, growth rate of the tumor, resistant mechanism for certain cytotoxic killing) or to extratumor factors of the patients (eg, bioavailability of antibody, status of effector cells) is unclear. In ADCC, rituximab binds to CD20 and then bridges the effector cells, such as NK cells and macrophages, via the FcγR on these effector cells. The cells then become activated and kill the antibody-coated tumors.[10,18] The effectiveness of this process may depend on how well the effector cells are activated after the

Table 4. Clinical Response to Rituximab Therapy According to FcγRIIa Polymorphism

	H/H			H/R			R/R			R Carrier*			
	No. of Patients With Response	No. of Patients Assessable	%	No. of Patients With Response	No. of Patients Assessable	%	No. of Patients With Response	No. of Patients Assessable	%	No. of Patients With Response	No. of Patients Assessable	%	P†
1-3 months, objective response‡	16	20	80	27	43	63	13	24	54	40	67	60	.116
6 months, objective response	16	20	80	19	42	45	7	19	37	26	61	43	.005
9 months, objective response	14	20	70	13	39	33	5	17	29	18	56	32	.004
12 months, objective response	11	20	55	10	37	27	4	17	24	14	54	26	.027

Abbreviations: FcγRIIa, immunoglobulin G fragment C receptor IIa; H, histidine allele; R, arginine allele.

*Combination of H/R and R/R genotypes.

†Two-sided Fisher's exact test, comparing H/H to R carrier.

‡Includes partial response and complete response or complete response unconfirmed.

engagement of FcγR with antibody-coated target cells, and how sensitive the target cells are to the cytotoxic process.

Although primary lymphoma cells had been found to be sensitive to rituximab-mediated ADCC,[8] the relationship between tumor susceptibility to ADCC and response to treatment has not been reported. In this study, we examined whether the susceptibility of pretreatment tumor cells to ADCC differs between patients who responded to rituximab treatment and patients who did not. To compare different patients, we used mononuclear cells isolated from a healthy donor as the universal effector. This in vitro assay showed a wide range of rituximab-mediated ADCC in all three response groups (Fig 1). However, it failed to show a correlation with the clinical outcome. In addition, the tumor's susceptibility to ADCC did not correlate with the duration of clinical responses, nor did it correlate with the level of surface CD20.

Both NK cells and macrophages mediate ADCC after the engagement of certain FcγRs by the IgG. Three classes of FcγR are found on these effector cells that regulate their activation: FcγRIIa (CD32) and FcγRIIIa (CD16) activate, and FcγRIIb (CD32) inhibits activation. FcγRIIIa is expressed on both NK cells and macrophages, whereas FcγRIIa and IIb are found only on macrophages. Recently, a polymorphism of FcγRIIIa was identified at position 158 with either a V or F residue.[12] The significance of this bimorphic allotype is two-fold: First, FcγRIIIa of 158 V allele binds human IgG1 better than does the FcγRIIIa of 158 F allele.[16] Second, this increased binding of 158 V allele also translates to enhanced activation of effector cells and better ADCC.[13,19]

In this study, we confirmed the observation of Cartron et al[11] that 158 V/V genotype is associated with higher response rate to rituximab treatment. However, there were some differences between our two studies. First, the response rate in our patient group was lower than that in the previous report, especially at 12 months after treatment. This is consistent with previous observations of a lower response rate when rituximab is used as second-line treatment.[20,21] In addition, our patients probably had higher tumor burden because 53% of them had bulky (≥ 5 cm) disease compared with the previous study in patients with nonbulky disease. Second, although F carriers (V/F and F/F) showed a significantly lower response rate, the response rate in patients with 158 F/F was slightly higher than that in patients with 158 V/F. The biologic explanation of this phenomenon is unclear, given that patients with 158 V/F would be expected to have an intermediate response rate. Third, consistent with the previous report, we detected a difference between 158 V/V and

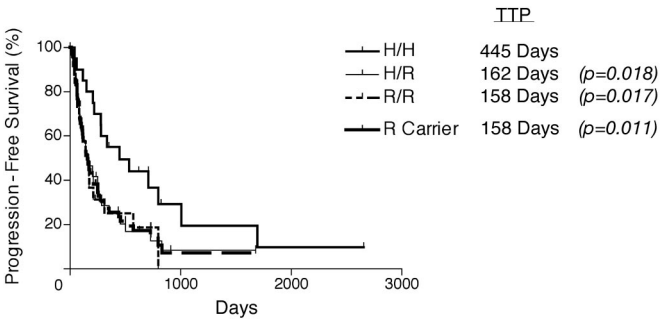

Fig 3. Kaplan-Meier estimates of progression-free survival (PFS) by immunoglobulin G fragment C receptor IIa (FcγRIIa) 131 histidine (H)/arginine (R) polymorphism. PFS curves were plotted by FcγRIIa 131 H/R genotype on all 87 patients. R carriers represent patients with either 131 H/R or 131 R/R genotype. TTP, median time to progression.

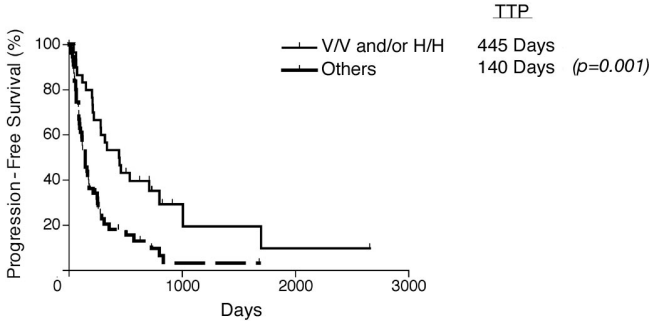

Fig 4. Progression-free survival (PFS) by immunoglobulin G fragment C receptor IIIa (FcγRIIIa) 158 valine (V)/phenylalanine (F) and FcγRIIa 131 histidine (H)/arginine (R) polymorphisms. PFS curves were plotted by FcγRIIIa 158 V/F and FcγRIIa 131 H/R genotype. Others represent patients without either FcγRIIIa 158 V/V or FcγRIIa 131 H/H genotype. TTP, median time to progression.

Table 5. Analysis of FcγRIIa and FcγRIIIa Polymorphism

		FcγRIIIa					
		V/V		F Carrier		V/F	F/F
FcγRIIa	No. of Patients	No. of Patients	%	No. of Patients	%	No. of Patients	No. of Patients
H/H	20	3	15	17	85	14	3
H/R	43	8	19	35	81	16	19
R/R	24	2	8	22	92	10	12

Abbreviations: FcγRIIa, immunoglobulin G fragment C receptor IIa; FcγRIIIa, immunoglobulin G fragment C receptor IIIa; H, histidine allele; R, arginine allele; V, valine allele; F, phenylalanine allele.

F carrier. However, one interesting observation in this study is that the difference became more pronounced after longer times from the treatment. The antibody is known to persist for up to 6 months, and its effect may be cumulative.

The most unexpected result came from the analysis of FcγRIIa polymorphism. The Allele of 131 H/H binds to human IgG2 better than that of 131 R/R. However, no significant difference in the affinity of these two allelic forms for human IgG1 has been noted.[22] Therefore, it was unexpected to find a higher rituximab response rate associated with 131 H/H genotype (Table 4). Similar to the FcγRIIIa 158 V/F polymorphism, we did not observe a gene dosage effect of the 131 H allele. Instead, the response rate in patients with 131 H/R was similar to that of 131 R/R at 6, 9, and 12 months. The biologic explanation of this observation is not clear. The association between FcγRIIa 131 H/H and higher response rate was not a result of a linkage disequilibrium of FcγRIIIa 158 V/F polymorphism (Table 5). There is a random distribution of combinations of variant genotypes of FcγRIIa and FcγRIIIa in the normal population.[23]

The FcγRIIa 131 H/R polymorphism is an independent predictive factor for clinical response: In the subgroup of patients with 158 F carrier, FcγRIIa 131 H/H genotype was associated with higher response rate at 6, 9, and 12 months (H/H = 76% *v* R carrier = 34%, $P = .004$ at 6 months; H/H = 65% *v* R carrier = 26%, $P = .007$ at 9 months; H/H = 47% *v* R carrier = 18%, $P = .026$ at 12 months). Furthermore, all three patients with both 158 V/V and 131 H/H genotypes had long-lasting remissions (Table 6). Patients with 158 V/V and/or 131 H/H genotypes showed a higher response rate and a longer remission than did patients without either of these two genotypes (Table 6). Lastly, the logistic regression analysis showed that the 158 V/V and 131 H/H were independent predictive factors for response at 6, 9, and 12 months. The report of Cartron et al[11] also analyzed the FcγRIIa 131 H/R polymorphism and concluded that the FcγRIIa polymorphism did not influence the clinical response. However, it is important to point out that Cartron et al analyzed a smaller group of patients (N = 45) and scored the clinical responses only at 1 and 12 months. In this study, the most prominent differences were observed at 6 and 9 months (Table 4).

The biologic explanation of the association between FcγRIIa 131 H/R polymorphism and rituximab response is not clear. Several variants of human IgG1 have been reported to have differential affinity to the 131 H/H and 131 R/R allotypes. In this study, three variants had higher affinity to 131 R/R and one bound 131 H/H more effectively.[13] It is possible that other yet unidentified polymorphic genes are linked to the 131 H/R locus and are responsible for the association with response rate.

Our data support a model showing that ADCC mediated through Fc receptors plays an important role in the antitumor effect of rituximab. Therefore, it may be possible to enhance the efficacy of rituximab, and possibly other mAbs, by engineering the Fc portion of the antibody to increase its binding to these activating FcγRs. Recently, trastuzumab (anti-HER2/*neu*) with re-engineered Fc portion was generated and showed enhanced ADCC.[13] The clinical efficacy of these trastuzumab analogues has yet to be tested. The inhibitory FcγR has also been shown to play a role in rituximab's antitumor effector in an animal model.[6] A recent study reported a polymorphism of isoleucine and threonine at position 232 of FcγRIIb involving the transmem-

Table 6. Clinical Response to Rituximab Therapy According to FcγRIIa and FcγRIIIa Polymorphism

	Both 158 V/V and 131 H/H (n = 3)			Either 158 V/V or 131 H/H (n = 27)			Others (n = 57)			
	No. of Patients With Response	No. of Patients Assessable	%	No. of Patients With Response	No. of Patients Assessable	%	No. of Patients With Response	No. of Patients Assessable	%	*P**
1-3 months, objective response†	3	3	100	22	27	81	31	57	54	.009
6 months, objective response	3	3	100	21	27	78	17	50	34	.0001
9 months, objective response	3	3	100	17	26	65	12	47	26	.0003
12 months, objective response	3	3	100	14	26	54	8	45	18	.0004

Abbreviations: FcγRIIa, immunoglobulin G fragment C receptor IIa; FcγRIIIa, immunoglobulin G fragment C receptor IIIa; V, valine allele; H, histidine allele.

*Two-sided Fisher's exact test, comparing 158 V/V and/or 131 H/H to others.

†Includes partial response and complete response or complete response unconfirmed.

Table 7. Prognostic Factors for Clinical Response: Logistic Regression Analysis

Characteristic	1-3 Months			6 Months			9 Months			12 Months		
	OR*	95% CI	P†	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
158 V/V	12.25	1.35 to 111.16	.026	8.48	1.54 to 46.60	.014	7.94	1.59 to 39.76	.012	17.14	2.94 to 100.18	.002
131 H/H	2.96	0.85 to 10.35	.090	8.03	2.13 to 30.21	.002	6.26	1.86 to 21.06	.003	7.25	1.89 to 27.84	.004
Stage III versus IV	0.66	0.19 to 2.22	.498	1.01	0.28 to 3.62	.984	0.62	0.16 to 2.40	.486	0.78	0.16 to 3.85	.759
Age ≥ 60 years	3.08	0.57 to 16.76	.193	2.62	0.57 to 12.12	.217	1.73	0.35 to 8.53	.500	4.22	0.73 to 24.25	.107
Prior transplant therapy	0.86	0.30 to 2.43	.772	0.55	0.17 to 1.73	.304	1.24	0.39 to 3.95	.717	2.18	0.56 to 8.45	.261
Bulky disease	1.81	0.54 to 6.05	.336	0.62	0.17 to 2.27	.470	0.68	0.18 to 2.53	.563	0.47	0.11 to 2.14	.333
≥ 2 extranodal sites of disease	1.22	0.30 to 4.89	.784	0.32	0.06 to 1.69	.181	0.57	0.11 to 2.84	.489	0.23	0.03 to 1.87	.170

Abbreviations: OR, odds ratio; V, valine allele; H, histidine allele.

*Relative odds of response to rituximab treatment.

†Two-sided; considered statistically significant for $P < .05$.

brane domain of the protein.[24] The biologic significance of this polymorphism is yet to be studied.

Both FcγRIIa and FcγRIIIa are expressed on the surface of dendritic cells and are believed to be involved in phagocytosis, antigen uptake, and presentation.[25] Although enhanced tumor immunity by targeting FcγR on dendritic cells has been demonstrated in mice,[26-28] the role of mAb therapy in promoting dendritic cell function in humans is not known. Recently, antibody-coated myeloma cells or lymphoma cell lines were shown to facilitate the cross-priming of tumor antigens by human dendritic cells in vitro.[29-31] In these studies, the antibodies coating the tumors were thought to engage the FcγR on dendritic cells, which leads to uptake of tumor antigens for presentation to T cells. It will be of great interest to determine if antitumor T-cell immune responses are generated after rituximab therapy. This might account for the long-lasting antitumor effect that can occur after rituximab therapy.

Now that the importance of FcγRIIa and FcγRIIIa polymorphisms has been established, it will be important to analyze these polymorphisms in future clinical trials using rituximab or other mAb therapy. It will also be interesting to study the influence of these two FcγR polymorphisms in patients treated with rituximab combined with chemotherapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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