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ORIGINAL REPORT

Clinical Outcome of Lymphoma Patients After Idiotype Vaccination Is Correlated With Humoral Immune Response and Immunoglobulin G Fc Receptor Genotype

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A B S T R A C T

Purpose
The unique immunoglobulin idiotype (Id) expressed by each B-cell lymphoma is a target for immunotherapy. Vaccination with Id induces humoral and/or cellular anti-Id immune responses. However, the clinical impact of these anti-Id immune responses is unknown. We and others have previously reported that immunoglobulin G Fc receptor (FcγR) polymorphisms predict the clinical response of lymphoma patients to passive anti-CD20 antibody infusions. In this study, we tested whether anti-Id immune responses or FcγR polymorphisms associate with clinical outcome of patients who received Id vaccination.

Patients and Methods
We analyzed 136 patients with follicular lymphoma who had received Id vaccination. The anti-Id immune responses were measured and FcγRIIIa and FcγRIIa polymorphisms were determined and correlated with clinical outcome for these patients.

Results
Patients who mounted humoral immune responses had a longer progression-free survival (PFS) than those who did not (8.21 *v* 3.38 years; $P = .018$). Patients with FcγRIIIa 158 valine/valine (V/V) genotype also had a longer PFS than those with valine/phenylalanine (V/F) or phenylalanine/phenylalanine (F/F) genotypes (V/V, 8.21 *v* V/F, 3.38 years; $P = .004$; *v* F/F, 4.47 years; $P = .035$). Multivariate analysis using the Cox proportional hazards model showed that V/V genotype and humoral immune responses were independent positive predictors for PFS.

Conclusion
This study is the first to identify the predictive value of FcγR polymorphism on clinical outcome in patients who received active immunotherapy with tumor antigen vaccines. Our results imply that the antibodies induced against a tumor antigen are beneficial and that Fcγ*R*-bearing cells mediate an antitumor effect by killing antibody-coated tumor cells.

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INTRODUCTION

Although chemotherapy and radiotherapy are used to treat B-cell non-Hodgkin's lymphoma, they carry significant side effects and are usually not curative.[1] As an alternative, immunotherapies are being developed to target specific tumor antigen(s). One of the most successful examples is the anti-CD20 monoclonal antibody (mAb), rituximab. Another example is active vaccination of lymphoma patients against antigens expressed by their tumor. Each B cell expresses an immunoglobulin, which contains unique variable region sequences in the heavy and light chain. During tumorigenesis, these unique sequences are maintained by the malignant clone, and the proteins they encode (idiotype, Id) represent tumor-specific antigens. The Id

protein can be isolated from the lymphoma cells and formulated into a vaccine.[2]

Lymphoma patients vaccinated with Id protein make humoral and cellular anti-Id immune responses to a variable degree, depending on the nature of the vaccine. In one study, patients who developed anti-Id immune responses had longer progression-free survival (PFS) and longer overall survival than patients who did not.[3] However, it is not yet known which component of the immune responses (humoral *v* cellular) is associated with the better outcome. In this report, we determined the predictive value of humoral anti-Id immune responses on the clinical outcome of a large patient population who received Id vaccination and who have had a long-term follow-up. Because FcγR allotypes are known to affect the ability of macrophages and natural killer cells to mediate antibody-dependent cellular cytotoxicity (ADCC),[4,5] we further determined the effect of FcγR polymorphism on the clinical outcome of these vaccinated patients.

PATIENTS AND METHODS

Id Vaccination Studies

This study included 136 patients who were treated with Id vaccination between 1988 and 2000. There were 81 cases of follicular small cleaved, 52 cases of follicular mixed, and three cases of follicular large-cell lymphoma. All patients received induction chemotherapy to achieve a maximal clinical response before vaccination. Of the 136 patients, 134 were in their first remission and two were in subsequent remission. Patients were staged with computed tomography scans before vaccination. Vaccinations were initiated at least 2 months after completion of chemotherapy.

In 118 cases, Id proteins were isolated using the rescue hybridoma method, whereas molecular rescue was used in 18 cases.[2,6,7] In all cases, keyhole limpet hemocyanin (KLH) was coupled to the Id proteins using glutaraldehyde to make the final Id vaccines.[2] During the vaccination, 86 patients received chemical adjuvant, 18 patients received cytokine adjuvant (granulocyte-macrophage colony-stimulating factor [GM-CSF]), and 32 patients had Id protein-pulsed dendritic cells.[6,7] The vaccination was composed of four to five injections, usually monthly depending on the specific protocols. Routine follow-up after vaccination was conducted with physical examinations, blood counts, and computed tomography scans. All vaccination studies were conducted according to institutional review board–approved protocols, and informed consent was obtained from all patients for the use of tissue samples and the analysis of clinical information.

Humoral Immune Response Assessments

Enzyme-linked immunosorbent assay was used to analyze sera for anti-Id antibodies as described.[3,7] Autologous tumor Id or irrelevant Id proteins were immobilized onto plates. Prevaccine and postvaccine sera were serially diluted and allowed to bind to the target Id proteins. The bound antibodies were then detected with either polyclonal goat antihuman immunoglobulin (Ig)G, antihuman IgM, or anti-Ig light chain. A specific humoral anti-Id immune response was considered positive when a four-fold increase in anti-Id titer was found in postvaccine serum compared with the prevaccine serum and to the isotype-matched irrelevant Id proteins used as specificity controls.

Cellular Immune Response Assessments

T-cell proliferation assays were performed as described.[2,3,6] Peripheral-blood mononuclear cells were cultured in quadruplicate in media alone or with tumor Id or irrelevant Id proteins. Incorporation of [^{3}H]thymidine was measured after an overnight pulse (1 μCi) on day 5. A response was interpreted as positive when the incorporation of more than twice the background (media alone) was observed on two or more occasions.

Analysis of FcγRIIIa and FcγRIIa Polymorphisms

Genomic DNA was prepared from tumor cells or peripheral-blood mononuclear cells using a DNA extraction kit (QIAGEN, Valencia, CA). In six cases, DNA was prepared from the serum as described.[8] Genotyping of FcγRIIIa 158 V/F and FcγRIIa 131 H/R polymorphism was performed using the TaqMan technology on an ABI Prism 7900HT Sequence Detector System (Applied Biosystems, Foster City, CA). Probes and primers were obtained from Applied Biosystems. In brief, FcγRIIIa and FcγRIIa-specific primer pairs flanking the polymorphic sites were used for amplification of genomic DNA. Probes specific to FcγRIIIa 158 V and FcγRIIa 131 H alleles were labeled with VIC fluorescent at the 5′ end and with nonfluorescent quencher at the 3′ end. Probes specific to FcγRIIIa 158 F and FcγRIIa 131 R alleles were labeled with FAM fluorescent at the 5′ end and with nonfluorescent quencher at the 3′ end. Polymerase chain reactions were prepared in a final volume of 5 μL, with 1X TaqMan Universal Polymerase Chain Reaction Master Mix, 4 ng of input DNA, primer pairs, and two probes (VIC- and FAM-labeled) for either FcγRIIIa or FcγRIIa polymorphisms. Polymerase chain reaction consisted of 50°C initiation for 2 minutes and AmpliTaq Gold activation at 95°C for 10 minutes, followed by 92°C for 15 seconds and 60°C for 1 minute, for 40 cycles. Each sample was set up as duplicate. The FcγR genotypes was determined using Allelic Discrimination protocol in SDS software provided by Applied Biosystems.

Statistical Analysis

Differences in the percentage of anti-Id immune responses were tested by single-factor analysis of variance test. The median time to progression (TTP) and difference in the PFS were determined using the Kaplan-Meier estimation and log-rank statistic (PRISM for Macintosh, GraphPad Software, San Diego, CA). Using the Cox proportional hazards model, an analysis including age (≥ or < 60 years), stage (III *v* IV), presence of clinical symptoms, male sex, humoral or cellular anti-Id immune response, and FcγRIIa and FcγRIIIa genotype was used to identify independent prognostic variables influencing the PFS (StatView 5.0.1, SAS Inc, Cary, NC).

RESULTS

Anti-Idiotype Immune Response and Clinical Outcome

One hundred thirty-six consecutive patients were vaccinated according to different protocols with a custom idiotype vaccine derived from their own tumor. Idiotype-specific humoral and cellular immune responses were detected in 48 patients (35%) and 27 patients (20%),

respectively. The frequency of anti-Id antibody (Ab) response did not differ between the two methods used for vaccine production (rescue hybridoma, 32%, *v* molecular rescue, 56%; $P = .07$) or between the different immunologic adjuvants tested (chemical, 35%; dendritic cell, 25%; cytokine, 56%; $P = .09$). However, patients who received a chemical adjuvant developed fewer cellular immune response than those who received either dendritic cells or a cytokine adjuvant (chemical, 9%; dendritic cell, 34%; cytokine, 44%; $P = .0002$; Table 1).

The patients who developed anti-Id Abs had longer PFS after the last chemotherapy than those who did not. The estimated PFS at 5 years was 61% for patients with anti-Id Abs and 38% for patients without anti-Id Abs, with median TTP estimates at 8.21 and 3.38 years for the two groups, respectively ($P = .018$; Fig 1). In some cases, we detected an increase in Ab titer that was not specific, having equal reactivity with irrelevant Id proteins. The PFS at 5 years was 33% for patients with nonspecific Abs, with median TTP of 2.58 years. Therefore, the association with favorable clinical outcome was limited to the induction of a specific Ab response. Within the group of 48 patients who developed specific Abs, the Ab titer determined by enzyme-linked immunosorbent assay did not correlate with TTP.

In contrast, development of a cellular anti-Id immune response had no relationship with PFS. The PFS at 5 years was 36% for patients with cellular immune responses and 49% for patients without cellular immune responses, with median TTP of 2.47 and 4.92 years, respectively ($P = .312$).

FcγRIIIa 158 V/F Polymorphism and Clinical Outcome

The results described above are consistent with a therapeutic effect of vaccination-induced Abs. One possible mechanism of action for anti-Id Abs is through ADCC, which requires the activation of killer cells via their FcγRs. Recently, we and others have found an association between FcγR genotypes and the response to rituximab therapy.[9,10] Presumably, the mAb mediates ADCC more efficiently in patients with favorable genotypes, because these FcγRs have higher affinity to the Fc of the Ab. We therefore tested

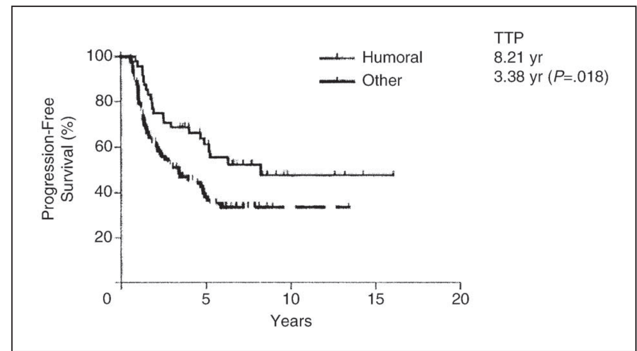

Fig 1. Kaplan-Meier estimates of progression-free survival by humoral anti-idiotype (Id) immune response. Progression-free survival curves were plotted by humoral anti-Id immune response. Humoral represents patients with specific anti-Id antibodies. Other represents patients without anti-Id antibodies. TTP, median time to progression.

whether FcγR polymorphisms correlated with the clinical outcome in Id vaccinated patients.

For the FcγRIIIa, 20 (15%) were homozygous valine/valine (158 V/V), 60 (44%) were heterozygous valine/phenylalanine (158 V/F), and 56 (41%) were homozygous phenylalanine/phenylalanine (158 F/F). The three groups were not different in terms of age at the time of therapy, fraction of patients with clinical symptoms, or time interval between diagnosis and therapy (Table 2). Additionally, the distribution of genotypes did not differ between patients who received Id proteins produced from the two methods, nor did they differ between patients receiving different immunologic adjuvants (data not shown). The estimated PFS at 5 years was 77% for patients with 158 V/V, 38% for 158 V/F, 48% for 158 F/F, and 41% for F carriers, with median TTP of 8.21, 3.38, 4.47, and 3.38 years for each group, respectively. The PFS curve in patients with 158 V/F was similar to that in patients with 158 F/F. For that reason, we grouped 158 V/F and 158 F/F together as the F carrier for statistical analysis. The PFS estimate of patients with 158 V/V was significantly longer than that of F carriers ($P = .009$; Fig 2A).

We further determined whether FcγRIIa polymorphism was correlated with the outcome after Id vaccination.

Table 1. Immune Response to Idiotype Protein After Vaccination

Adjuvant	No. of Patients	Humoral No.	Humoral %	Cellular No.	Cellular %	Both (No.)
Chemical*	86	30	35	8	9	6
Dendritic cell	32	8	25	11	34	1
Cytokine	18	10	56	8	44	5
Total	136	48	35	27	20	12

*Chemical adjuvants used: syntex adjuvant formulation (SAF), incomplete SAF (ISAF), QS-21 (Antigenics, New York, NY), and SBAS-2 (GlaxoSmithKline Biologicals, Rixensart, Belgium). Cytokine: granulocyte-macrophage colony-stimulating factor.

Table 2. Characteristics of the Patients According to Their Fcγ Receptor Polymorphism

Characteristic	FcγR IIIa Polymorphism			All Patients (N = 136)	FcγR IIa Polymorphism		
	V/V (n = 20)	V/F (n = 60)	F/F (n = 56)		H/H (n = 35)	H/R (n = 59)	R/R (n = 42)
Sex, male/female	12/8	29/31	27/29	68/68	17/18	27/32	24/18
Age, years							
Mean	47	47	46	46	46	45	47
SD	14.1	10.1	9.1	10.4	10.9	9.9	9.9
Pathology							
FSC	13	32	36	81	19	32	30
FM	6	27	19	52	15	26	11
FLC	1	1	1	3	1	1	1
Stage							
III	7	19	16	42	16	18	8
IV	13	41	40	94	19	41	34
Clinical symptoms*	2	8	4	14	1	10	3
Time between diagnosis and treatment, months							
Mean	8	15	12	13	18	10	13
SD	7	27	16	21	26	10	27

Abbreviations: FSC, follicular small cleaved; FM, follicular mixed; FLC, follicular large cell.
*Fever, night sweats, weight loss.

Of the 136 patients, 35 patients (26%) were homozygous histidine/histidine (131 H/H), 59 patients (43%) were heterozygous histidine/arginine (131 H/R), and 42 patients (31%) were homozygous arginine/arginine (131 R/R; Table 2). In contrast to FcγRIIIa polymorphism, the FcγRIIa 131 H/R polymorphism had no correlation with PFS (Fig 2B). The estimated PFS at 5 years was 49% for patients with 131 H/H, 48% for 131 H/R, 43% for 131 R/R, and 46% for R carrier, with median TTP of 4.83, 4.92, 3.41, and 4.68 years, respectively.

It was possible that the association of FcγRIIIa genotype with clinical outcome would also be found for patients who received only chemotherapy without vaccine therapy. To examine this, we analyzed a total of 158 independent patients with follicular lymphoma who were treated with chemotherapy alone and whose samples were also available for genotyping. Of these 158 patients, the estimated PFS at 2 years was 32% for patients with 158 V/V, 35% for patients with 158 V/F, 40% for patients with 158 F/F, and 38% for F carriers, with median TTP of 1.65, 1.30, 1.53, and 1.44 years, respectively. The PFS estimate of patients with 158 V/V was not different from that of other patients (V/V *v* V/F; $P = .642$; V/V *v* F/F, $P = .435$; V/V *v* F carrier; $P = .805$). Similarly, the FcγRIIa 131 H/R polymorphism had no impact on the clinical outcome in this chemotherapy-treated patient group (data not shown).

Because the Ab response and the FcγRIIIa genotype are distinct immunologic variables, we next analyzed the correlation of FcγRIIIa 158 V/V genotype and the anti-Id Abs separately and together with clinical outcome. The PFS estimate of patients with anti-Id Abs and/or 158 V/V (total of 64 patients) was significantly longer ($P < .0001$; Fig 3). In a multivariate analysis, FcγRIIIa 158 V/V genotype and anti-Id Abs emerged as two independent positive predictors for longer PFS, whereas none of the others was identified as a predictive factor (Table 3).

DISCUSSION

Immunotherapy using Id vaccination in low-grade B-cell lymphoma has been under development for more than 15 years. Previous studies have focused on producing Id proteins more efficiently and developing more potent adjuvants to increase the frequency of immune responses. However, the clinical impact of the specific type of anti-Id immune responses have not been fully assessed, and the methods for measuring the immune response have not been validated. To address these questions, we analyzed a group of patients who had received Id vaccination and had long-term follow-up. Although these patients had received Id vaccination under different protocols, they were treated in a similar way. They all received induction chemotherapy to reduce tumor burdens, followed by Id vaccination after a short recovery period. The vaccines were composed of custom-made Id protein coupled to KLH along with immunologic adjuvant. Overall, 47% of patients developed anti-Id immune responses (humoral and/or cellular), which was similar to our previous report on a subset of these same patients.[3] The development of anti-Id Abs was not influenced by the Id protein production methods or by the immunologic adjuvants (Table 1). In contrast, the

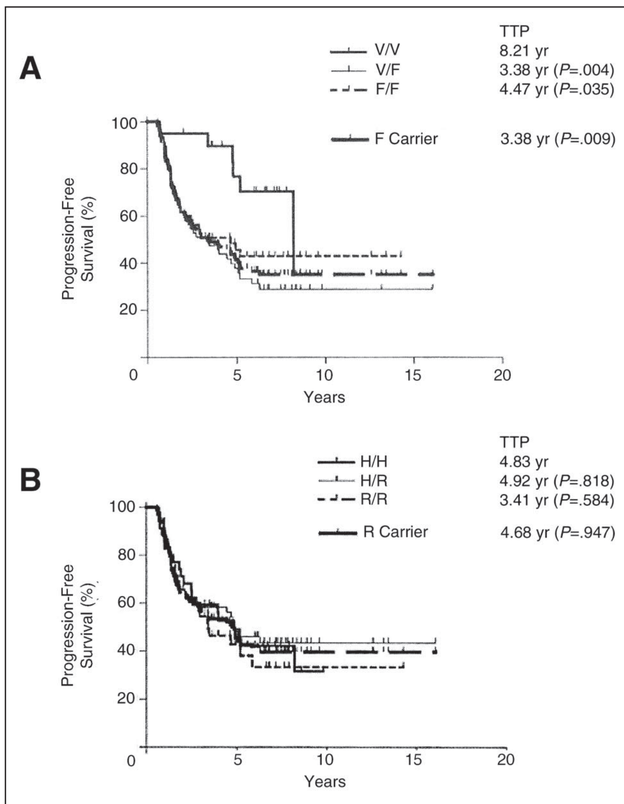

Fig 2. Kaplan-Meier estimates of progression-free survival by FcγR polymorphisms. Progression-free survival curves were plotted by (A) FcγRIIIa 158 valine/phenylalanine (V/F) and (B) FcγRIIa 131 histidine/arginine (H/R) genotype. F carriers represent patients with either 158 V/F or 158 phenylalanine/phenylalanine genotype. R carriers represent patients with either 131 H/R or 131 R/R genotype. TTP, median time to progression.

development of cellular immune responses was greatly enhanced by using dendritic cells or GM-CSF. This finding is consistent with the notion that dendritic cells play the principal role in priming T-cell response.[3,6,11,12] Also, the high

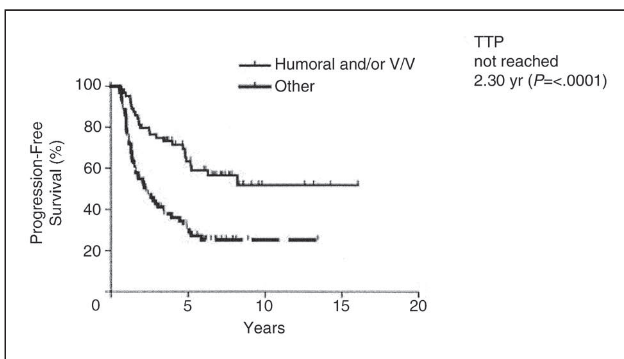

Fig 3. Progression-free survival by humoral anti-idiotype (Id) immune response and FcγRIIIa 158 valine/phenylalanine (V/F) polymorphism. Progression-free survival curves were plotted by humoral anti-Id immune responses and/or FcγRIIIa 158 V/F genotype. Other represents patients without either anti-Id antibodies or FcγRIIIa 158 valine/valine genotype. TTP, median time to progression.

Table 3. Prognostic Factors for Freedom From Progression: Cox Proportional Hazards Model

	Relative Benefit*	95% CI	P†
158 V/V	4.16	1.75 to 9.89	.0013
131 H/H	0.73	0.42 to 1.27	.267
Humoral immune response	2.31	1.38 to 3.85	.0015
Cellular immune response	0.72	0.41 to 1.25	.243
Stage III *v* IV	0.63	0.37 to 1.07	.088
Clinical symptoms‡	1.17	0.55 to 2.49	.689
Age ≥ 60 years	0.88	0.42 to 1.87	.746
Sex, male	0.71	0.44 to 1.12	.140

Abbreviations: V/V, valine/valine; H/H, histidine/histidine.
*Relative benefit: to have longer freedom from progression from last chemotherapy.
†All P values are two-sided and considered to be statistically significant for $P < .05$.
‡Fever, night sweats, weight loss.

induction rate of T-cell responses has been demonstrated in another Id vaccination trial using GM-CSF.[13]

The anti-Id Ab is predicted to be critical for the effect of Id vaccines. Animal models have demonstrated that the efficacy of Id vaccination depends on the Ab response.[14-17] Moreover, infusion of custom-made anti-Id mAbs induced tumor regression in a high fraction of B-cell lymphoma patients, some of which have remained tumor-free for more than 10 years.[18] Indeed, patients who developed detectable anti-Id Abs had longer PFS (Fig 1). However, there are major differences between anti-Id Abs induced by active vaccination and the passively infused anti-Id mAbs. First, vaccinated patients received induction chemotherapy to reduce their tumor burden before vaccination. Therefore, the clinical effects of Id vaccination must be determined by TTP as in this study, whereas the effect of passive Ab infusions has been measured by tumor regression. Second, the passively infused anti-Id mAbs have a limited residence in the body. In contrast, vaccination-induced anti-Id can persist for long periods of time. In this study, only specific anti-Id Ab responses were associated with a superior outcome, whereas nonspecific Ab had no impact. These findings serve to validate our anti-Id Ab assay and its criteria for positivity in relation to outcome. However, the Ab titer was not correlated with clinical outcome in patients who developed positive Ab response. This threshold effect of Ab response is consistent with several published results in animal models.[14-16]

It was surprising that in our study, the cellular immune response, as measured, was not associated with better outcome. There are several possible explanations. First, protein vaccines tend to induce Abs better than cellular immune responses.[19] Second, our cellular immune responses were determined by cell proliferation in response to Id protein. This assay measures mainly the $CD4^+$ as opposed to the $CD8^+$ T-cell response, which is thought to be more

important in antitumor effects.[20,21] Third, it is possible that Ab has a more potent antilymphoma effect. Indeed, low-grade lymphoma has been shown to be very responsive to passive Ab therapy.[18,22] Nonetheless, antilymphoma cellular immune responses are believed to mediate clinically important effects in some Id vaccination patients.[13] In our own series, we have observed durable tumor regression in the absence of detectable anti-Id Abs while demonstrating T-cell response and tumor-specific cytotoxic responses.[6]

The mechanisms of antitumor action of anti-Id Abs are unknown. One possibility is through direct killing of lymphoma cells.[23,24] In a previous study, the passively infused anti-Id mAbs induced signal transduction in autologous tumor cells, and the degree of signaling correlated with clinical response.[23] These results suggested a direct effect of anti-Id–mediated signaling. Another possible mechanism is through ADCC. In this process, anti-Id Abs bind to the tumors and then bridge the effector cells via the FcγR on these cells. The effector cells then become activated and kill the Ab-coated tumors.[25,26] The effectiveness of ADCC may depend on the degree of activation of effector cells after FcγR engagement. Three classes of FcγR are found on these cells that regulate their activation: FcγRIIa (CD32a) and FcγRIIIa (CD16) activate, whereas FcγRIIb (CD32b) inhibits activation. FcγRIIIa is expressed on both natural killer cells and macrophages, whereas FcγRIIa and IIb are found only on macrophages. In addition to these three constitutively expressed FcγRs, FcγRI (CD64) is inducible by interferon gamma and GM-CSF on neutrophils and macrophages that could also mediate ADCC.[27,28] Recently, polymorphisms of FcγRIIIa and FcγRIIa were identified that affect their affinity for the Fc of Abs and probably the efficiency of ADCC. For FcγRIIIa, a valine residue at position 158 (158 V) binds to human IgG1 better than a phenylalanine residue (158 F).[29,30] For FcγRIIa, allele of histidine at position 131 (131 H) binds to human IgG2 better than that of arginine (131 R).[31] We recently found that FcγRIIIa 158 V/V and FcγRIIa 131 H/H are associated independently with a higher response rate to rituximab.[9] Here, we found that patients with FcγRIIIa 158 V/V had a longer PFS after Id vaccination (Fig 2). In fact, FcγRIIIa 158 V/V emerged as an independent predictor along with Ab response (Table 3). This observation has several important implications. First, ADCC plays a major role in the protective effect of Id vaccination. However, other FcγR-mediated processes cannot be excluded, such as FcγR-mediated antigen delivery and activation of dendritic cells.[32,33] Second, because the correlation with 158 V/V genotype was not limited to the patients who had detectable anti-Id Abs, it is conceivable that low-level anti-Id Abs had been induced in these patients that were below the sensitivity of our assay. It is also possible that antibodies against other tumor-specific antigens through cross-presentation have been induced after vaccination. In this case, these other antitumor antibodies would not be detected by our assay. Third, it is expected that 158 V/V patients who developed anti-Id Abs would do better than anti-Id Ab-positive patients who are F carriers. In our current study, only four patients with 158 V/V genotype had detectable anti-Id Abs. It is, therefore, difficult to find the difference with such a small number of patients. Although all patients in this study received chemotherapy before vaccination, the better outcome associated with FcγRIIIa 158 V/V genotype was specific to Id vaccination. There was no clinical impact of FcγRIIIa V/F polymorphism on patients who received chemotherapy alone (see Results). Consistent with previous reports on rituximab-treated patients,[9,10] our study also showed that patients with 158 V/F had a similar clinical outcome as patients with 158 F/F. However, the biologic explanation of this phenomenon is unclear, because patients with 158 V/F would be expected to have an intermediate clinical course. It is possible that a certain threshold for Fc*γR*-delivered signal is required to activate the effector cells fully, which can only be reached by signaling through the products of two V alleles.

The major difference in affinity between the 131 H and 131 R allele is in binding to the human IgG2.[31] Because the vaccination-induced Ab response was a polyclonal reaction, the anti-Id Abs should not be limited to certain isotype. Therefore, it was expected that the FcγRIIa polymorphism would affect the outcome. However, in contrast to our previous study on rituximab-treated patients, the FcγRIIa 131 H/R polymorphism had no clinical impact on these Id vaccine-treated patients. One possibility is that the majority of the induced anti-Id Abs was of IgG1 isotype. Bendani et al[13] have reported that all except one case of the Ab responders had anti-Id Abs of IgG1 subclass in their series. Although we did not routinely determine the isotype of anti-Id Abs, a few cases we tested showed Abs of multiple isotypes including IgG1 (data not shown).

Our data support a model that antitumor Ab-mediated ADCC plays an important role in the antitumor effect of Id vaccination. Therefore, it might be possible to improve the efficacy of Id vaccination by improving the Ab response. First, vaccination using Id proteins that are more close to native protein is preferable with the goal to induce Abs recognizing native Id on the tumor cells. Second, vaccine boosting may be warranted to maintain the high-titer of anti-Id Abs. Lastly, while rituximab gains popularity as first-line therapy for low-grade B-cell lymphoma, it should be recognized that the depletion of normal B cells by rituximab may interfere with future attempts to induce an Ab response against the tumor. Most patients experience decreases in the number of B cells and have impaired humoral immunity for 6 to 9 months after rituximab.[22,34] Therefore, it may be important to delay vaccination until after recovery of B cells. Our study implies a clinical benefit of an Ab response in a large patient group vaccinated against Id. The

analysis of FcγR polymorphism not only argues for the role of Abs, but also provides a way to predict who may benefit from Id vaccination and should be examined in Id vaccination trials that are now underway. Although most of the cancer immunotherapy using tumor antigen vaccines focuses on the induction of cytotoxic T cells,[35-37] our study clearly shows the importance of Ab response against tumor antigen. Although immunotherapies using antibodies or tumor antigen vaccination were first applied to hematologic malignancies, Ab therapies have now found a role in the treatment of a variety of solid tumors.[38-40] Therefore, therapeutic vaccine studies in patients with solid tumors should also consider the potential role of antitumor Abs in evaluating clinical outcome.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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