FcγR polymorphisms and clinical outcome in colorectal cancer patients receiving passive or active antibody treatment

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Abstract. Fcγ receptors (FcγRs) on effector cells are of importance for mediating antibody-dependent cellular cytotoxicity (ADCC). FcγRIIa158valine (V)/phenylalanine (F) and FcγRIIa131histidine (H)/arginine (R) polymorphisms have been shown to relate to prognosis in antibody-treated patients. The aim of the present study was to analyze the polymorphisms of both FcγRIIa and FcγRIIa in colorectal carcinoma (CRC) patients receiving either passively administered monoclonal antibodies (MAbs) or antibodies induced by carcinoembryonic antigen (CEA) vaccination. One hundred and thirty CRC patients were included. Thirty-eight patients received adjuvant treatment with an anti-EpCAM monoclonal antibody (edrecolomab) (n=17) or rCEA vaccination therapeutic cancer vaccine (TCV) (n=21) inducing anti-CEA IgG antibodies. Ninety-two patients had metastatic disease and received anti-EpCAM MAb based therapies. FcγR genotypes were analysed using genomic DNA and PCR. ADCC was tested in a standard 18 h Cr51 release assay. In all adjuvant-treated patients, FcγRIIa158V carriers (V/V and V/F) had a significantly better overall survival compared to F/F homozygous patients (p<0.05), FcγRIIa R carriers vs. H/H (p=0.05) as well as V and R carriers combined compared to the others (p<0.05). Similar findings were obtained when antibody and TCV-treated patients were analysed separately. No impact on the prognosis of FcγR polymorphisms was noted in advanced disease. FcγRIIa V carriers had a significantly higher ADCC activity compared to F/F patients (p=0.001).

Our model study might support the notion that FcγRIIa V carriers as well as FcγRIIa R carriers receiving adjuvant, passively or actively (TCV)-induced antibody treatment might have a better prognosis than the others. Prospective extended clinical trials are warranted to study the predictive/prognostic impact of FcγR polymorphisms in antibody-treated patients and might be a valuable biomarker to optimize antibody-based treatment strategies.

Introduction

Surgery remains the major therapeutic approach in colorectal carcinoma (CRC). The prognosis is still poor with an overall cure rate of ~50%. Systemic chemotherapy, both in the adjuvant setting, as well as in advanced disease has improved the outcome. However, there is a great need to improve systemic treatment.

Patients with CRC may spontaneously mount a tumor specific immune response which seems to relate to improved prognosis (1). Natural antibodies against tumor antigens such as the carcinoembryonic antigen (CEA) and epithelial cell adhesion molecule (EpCAM) have been reported to be associated with improved survival (2,3). Both EpCAM and CEA are considered promising target structures for immunotherapy with monoclonal antibodies (MAb) (4) as well as with therapeutic cancer vaccines (TCV) (5-8).

MAb alone or in combination with chemotherapy may induce tumor regression and prolong progression free and overall survival (4,9). Vaccination with CEA or EpCAM may induce specific antibodies and T cells (10,11,12). Patients receiving CEA TCV mounting high IgG antibody titers against CEA had a superior survival as compared to those with low titers or no CEA IgG antibodies (11).

Although MAb have been used in clinical trials for almost three decades, the underlying mechanisms of actions have not been fully elucidated. Antibody dependent cellular cytotoxicity (ADCC) is an important mechanism. The antibodies bind to the target structures on tumor cells and the Fc-part to Fc receptors (FcγRs) on effector cells. The effector cells are activated to kill the antibody-coated tumor cells by release of cytotoxic molecules (13). This mechanism of
action should be operating both for passively administered antibodies as well as for TCV-induced antibodies.

FcγRs on human leukocytes are of three distinct types: FcγRI, FcγRII, and FcγRIII. The latter two types can be further divided into FcγRIIα/FcγRIIB and FcγRIIa/FcγRIIib, respectively (14,15). FcγRI have high affinity for IgG and can bind monomeric IgG. FcγRII and FcγRIII have a weaker affinity for monomeric IgG and act more effectively when binding multimeric immune complexes. FcγRIIa and FcγRIIIa initiate a cytotoxic signal, while FcγRIIb mediates an inhibitory signal (16). FcγRIIIa is expressed on natural killer cells and macrophages, whereas FcγRIIa and FcγRIIb only on macrophages. The polymorphism of FcγR has been considered to be of importance for ADCC activity (14,15,17,18).

Follicular lymphoma patients treated with rituximab homozgyote for FcγRIIα131 H/H (histidine) and for FcγRIIIa158 V/V (valine) had a more favorable clinical outcome as compared to heterozygote or homozygote for arginine (R) or phenylalanine (F) (19). Similar results were observed in patient comparison to metastatic breast cancer treated with trastuzumab (17).

The objective of the current study was to assess the impact of FcγRIIIa and FcγRIIa polymorphisms on the prognosis of patients with CRC receiving either passive MAb treatment (adjuvant or advanced disease) or adjuvant TCV inducing antibodies.

Materials and methods

Patients. One hundred and thirty patients with CRC, 75 males and 55 females, with a median age of 64 years (range: 14-80 years) were included in the study. Details of patient characteristics and clinical effects have been described elsewhere (10,20-24). The study was approved by the Ethics Committee of the Karolinska Institute and informed consent was obtained from each patient.

Treatment protocols

A. Adjuvant treatment. Thirty-eight patients with no macroscopic disease following surgery, received adjuvant treatment either with MAb or TCV (Table I). The treatment protocols have been described previously (10,22).

1A. Recombinant CEA protein vaccination. Twenty-one patients were vaccinated seven times with a baculovirus-produced recombinant carcinoembryonic antigen (rCEA) protein (Protein Sciences Corp., Meriden, CT, USA) at four different dose levels over a 12-month period. Half of the patients received GM-CSF (Leucomax, Schering-Plough/Sandoz, Kenilworth, USA) (80 μg/day for 4 consecutive days) at each immunization (10).

2A. Murine anti-EpCAM (Edrecolomab) treatment. Seventeen patients with stage III colon cancer were enrolled into a randomised, open-label multi-centre study (22). Nine patients were randomised to receive treatment with the murine (IgG2A) anti-EpCAM monoclonal antibody (mMAb) edrecolomab (Centocor, Malvern, PA, USA) (25) alone (an initial 500 mg i.v. infusion following by four infusions of 100 mg every 4 weeks) (26). Eight patients received the combination of edrecolomab as above together with fluorouracil (425 mg/m² intravenous bolus daily for 5 days every 4 weeks for the first three cycles, then every 5 weeks for the last three cycles), and folic acid (20 mg/m² intravenous bolus daily for 5 days every 4 weeks for the first three cycles then every 5 weeks for the last three cycles) (26).

B. Anti-EpCAM mMAb treatment for metastatic disease. In an attempt to develop a MAb-based therapeutic regimen in CRC, patients with advanced disease were sequentially recruited to different protocols. In the present study, 92 patients were included (Table II) to four different treatment protocols. Two variants of an anti-EpCAM MAb were used: the murine anti-EpCAM mMAb edrecolomab (Centocor) (25), or the chimeric (IgG1) anti-EpCAM cMAb (Centocor) (27). In the treatment protocols A, B and C (see below), E. coli-derived human GM-CSF (Leucomax, Schering-Plough/Sandoz; specific activity: 1.1×10¹⁰ IU/mg protein), was also given with the aim to activate effector cells (28,29). The treatment protocols have been described in detail elsewhere (20,21,23,24) and are summarized below:

Protocols A and B. Anti-EpCAM MAb/GM-CSF trial. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was administered s.c. once daily for 10 consecutive days. At day 3 of a treatment cycle, edrecolomab (protocol A)
Recombinant human daily for five consecutive days. At day 4 and 5, 500 mg/m² Plough, Kenilworth, New Jersey, USA) was given s.c. once divided in 24 doses) were used (23). Thirty-six patients were one gram divided in four doses to a maximum of 12 gram increasing doses of edrecolomab alone (from a total dose of Protocol D. Twenty-one patients were included. After a 2-day rest, GM-CSF was administered s.c. once daily treatment cycle was repeated every 4th week until progression. Day 8-14. On day 10, edrecolomab was infused i.v. (21). The differentiation as follows: Poorly differentiated 26 28.3% Moderate differentiated 61 66.3% Well differentiated 5 5.4% Metastatic sites One 60 65.2% More than one 32 34.8% (n=12) (24) or the chimeric (protocol B) (n=23) anti-EpCAM MAb (20), was infused i.v. for 60 min. The treatment cycle was repeated every 4th week.

Protocol C. Anti-EpCAM MAbs GM-CSF/α-IFN/5-FU trial. Recombinant human α-IFN (Introna® (α-2b), Schering-Plough, Kenilworth, New Jersey, USA) was given s.c. once daily for five consecutive days. At day 4 and 5, 500 mg/m² of 5-fluorouracil (Fluracetyl® (5-FU), Nycomed, Lidingo, Sweden) was administered as a daily i.v. bolus injection. After a 2-day rest, GM-CSF was administered s.c. once daily days 8-14. On day 10, edrecolomab was infused i.v. (21). The treatment cycle was repeated every 4th week until progression. Twenty-one patients were included.

Protocol D. Anti-EpCAM mMAb (edrecolomab) alone trial. Increasing doses of edrecolomab alone (from a total dose of one gram divided in four doses to a maximum of 12 gram divided in 24 doses) were used (23). Thirty-six patients were included.

FcγRIIIa and FcγRIIa genotype analysis. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using a DNA isolation kit (Qiagen, Hilden, Germany). DNA concentration was measured by Nano Drop. Genotypes of FcγRIIIa158V (valine/phenylalanine) and FcγRIIa131H/R (histidine/arginine) polymorphisms were detected by a TaqMan SNP genotyping assay using a Real-time PCR 7900 device (Applied Biosystems, Stockholm, Sweden). Probes and primers were obtained from Applied Biosystems. Probes specific for the FcγRIIIa158V and FcγRIIa131H, alleles were respectively labeled with VIC fluorescent at the 5' end and with nonfluorescent quencher at the 3' end. Probes specific for FcγRIIIa158F and FcγRIIa131R, alleles were labeled with FAM fluorescent at the 5' end and with nonfluorescent quencher at the 3' end. Each DNA sample was prepared in a final volume of 5 μl, mixed with the same volume (5 μl) 1X TaqMan Universal genotyping Master mix, 4 ng of input DNA, primer pairs, and two probes (VIC and FAM labeled) for each pair of polymorphisms. PCR consisted of 50°C initiation for 2 min and AmpliTaq Gold activation at 95°C for 10 min, followed by 92°C for 15 sec and 60°C for 1 min, for 40 cycles. The FcγR genotypes were determined using Allelic Discrimination protocol in SDS (Sequence Detection System) software provided by Applied Biosystems.

Cytotoxicity assay. The method has been described in detail previously (24). Briefly, cytotoxicity was determined in a ⁵¹Cr-release assay using the EpCAM expressing human CRC cell line SW948 as target and PBMC as effector cells at an effector-to-target (E:T) cell ratio of 50:1. Cytotoxicity was measured by incubating effector and target cells with the anti-EpCAM murine or chimeric MAb for 18 h. Maximum release was determined by incubation of the target cells with 5% Triton-X. Spontaneous release was determined by incubation of ⁵¹Cr-loaded targets cells with medium alone. The percentage of cytolysis was calculated according to the formula: lysis (%): (release in sample—spontaneous release)/(maximal—spontaneous release) x100.

Statistical analysis. Survival estimates were calculated using the Kaplan-Meier method. Patients receiving adjuvant treatment were assessed for overall survival (OS), calculated from the date of initiation of adjuvant therapy to the date of death from any cause, or to the date of last follow-up when data were censored. Disease-free survival (DFS) was calculated from the date of start of TCV or MAb therapy to the first observation of disease relapse or death from any cause. If a patient had not progressed or died, DFS was censored at the time of last follow-up. Patients treated for metastatic disease, were assessed for OS calculated from the date of treatment start to the date of death from any cause (all patients were followed until death). Progression-free survival (PFS) was calculated from the date of treatment to the first observation of disease progression. Differences in OS, DFS and PFS for various FcγR genotypes were analysed using the log-rank test.

Cox’s proportional hazard model was used to define independent prognostic factors (genotypes and baseline clinicopathologic features, such as age, gender, AJCC stage, tumor site and differentiation). Analyses were done for the entire group of adjuvant-treated patients as well as for the two different adjuvant treatment protocols separately. In patients with metastatic disease, the analyses were done for the entire group, as there were no statistically significant differences in PFS or OS between the various treatment protocols (data not shown). Mann-Whitney U test was used to test the relationship between polymorphisms and cytotoxicity. All the analyses were performed using the SPSS statistical package (version 13.0).

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Results

The frequencies of the FcγRIIa and FcγRIIIa genotypes are shown in Table III.

Relation between FcγR genotypes and prognosis in adjuvant-treated patients

All adjuvant-treated patients. At the time of analysis, 20 patients were alive and 18 had died. The median overall survival (OS) time for the whole group was 302 weeks. Twenty patients had disease progression with a median DFS of 80 weeks (CI95%: 73-87 weeks). The median follow-up time for living patients was 316 weeks (CI95%: 118-511 weeks).

FcγRIIIaV158F polymorphism showed a significant association to OS. OS of patients with 158V/V and 158V/F (V carriers) has not been reached as compared to 236 weeks for patients homozygote for 158F/F (p=0.0460) (Fig. 1A). FcγRIIa R carriers (H/R and R/R) patients had also a better survival than H/H patients (p=0.058). Median OS for H/H patients was 238 weeks while for R carriers the corresponding figure has not been reached (Fig. 1B). Moreover, V and R carriers together had a better survival than the remaining patients (p=0.0345). Median OS for the remaining patients was 260 weeks, while for V and R carriers together the corresponding figure was not reached (Fig. 1C). The impact on OS of FcγRIIIa and FcγRIIa and other clinical characteristics in univariate analyses are shown in Table IV.

In a multivariate Cox regression analyses including age (above or below 60), gender, tumor site (colon and rectum), tumor differentiation stage (poorly, moderately and well differentiated), AJCC stage (stage I+II vs. stage III+IV), R carriers (R carriers or not) and V carriers (V carriers or not), FcγRIIIa V carriers (V/V, V/F) was the only independent predictor for overall survival (p=0.015) (Table V).

Figure 1. Overall survival of all patients receiving adjuvant treatment (n=38) in relation to FcγRIIIa and FcγRIIa polymorphism. (A) RIIIa V carriers (solid line) (V/V, V/F) (n=21) and F/F (dotted line) (n=17) (p=0.046). (B) RIIa R carriers (solid line) (R/R, H/R) (n=24) and H/H (dotted line) (n=14) (p=0.058). (C) RIIIa and RIIa V and R carriers (solid line) (n=13) and remaining patients (dotted line) (n=25) (p=0.0345).

FcγRIIIa V carriers (V/V, V/F) was the only independent predictor for overall survival (p=0.015) (Table V).

Adjuvant edrecolomab antibody alone treatment group. All patients in the edrecolomab alone group were in AJCC stage III. The relationship between FcγRIIIa polymorphisms and OS is shown in Fig. 2A. There was a tendency to better survival for V carriers as compared to F/F (p=0.126). Median survival for F/F homozygote patients was 98 weeks, while the corresponding time for V carriers was not reached. Comparing the group of patients being both FcγRIIIa V carriers and FcγRIIa R carriers with the remaining patients...
showed a clear tendency to better survival for those being both V and R carriers (p=0.0594) (Fig. 2B). Median survival for patients being both H/H and F/F homozygote was 98 weeks while median OS for combined V and R carriers was not reached. In a multivariate analyse (for factors see Table IV) no factor was shown to independently influence survival (data not shown).

Adjuvant rCEA vaccine treatment alone group. Median OS for FcγRIIa R carriers has not yet been reached as compared to 260 weeks for homozygous (131H/H) patients (p=0.0488) (Fig. 3). In a univariate analyses (Table VI), age and FcγRIIa R carriers versus H/H were significantly related to OS. Median OS for F/F homozygous patients was 236 weeks while that of V carriers has not been reached (p=0.21) (data not shown). There was however a highly statistical difference in OS when patients being both V and R carriers were compared to the remaining group of patients (p<0.0001) (Fig. 3B). In a multivariate analyses of factors for OS, FcγRIIIa V carriers were an independent factor for improved OS (p=0.036) as well as age <60 years (p=0.017) (data not shown).

MAb treatment in metastatic disease and the relation to FcγR polymorphism. In patients with advanced disease there was no relation between FcγRIIIa and FcγRIIa polymorphisms and response rate, PFS or OS, respectively, either analyzing the different treatment protocols separately or the whole group (data not shown).

ADCC in relation to FcγR polymorphism. ADCC was analyzed at diagnosis in 64 of the 92 patients with metastatic disease. FcγRIIIa V carriers had a significantly higher ADCC activity as compared to F/F homozygote patients (p=0.001)
There was however no relation between ADCC activity and response rate, PFS or OS, respectively (data not shown; ADCC was not tested in the adjuvant treatment group).

**Discussion**

The current study suggests that FcγRIIA and FcγRIIa polymorphisms might be a prognostic factor for adjuvant-treated CRC patients receiving passive antibody therapy or TCV inducing an antibody response. In antibody-treated patients, FcγRIIA V carriers as compared to F/F had an improved survival as well as FcγRIIa R carriers as compared to H/H. Moreover, the group of patients being both V and R carriers had a better prognosis. ADCC activity in vitro was significantly higher among FcγRIIA V carriers as compared to F/F patients. In metastatic disease no impact of FcγRIIA and FcγRIIa genotypes was noted.

The FcγRIIa and RIIa distribution frequency in the present study was consistent with previous studies in Caucasian patients with CRC (18,30). Our results on FcγRIIa are partly in line with some previous reports showing an association between the clinical effects of MAb treatment and FcγRIIa polymorphism. In lymphoma, the FcγRIIa158 V/V genotype was associated with a superior clinical outcome in rituximab-treated follicular, diffuse large B cell lymphoma and Waldenström macroglobulinemia patients compared to F carriers (19,31,32,33,34). However, there are also studies showing no relation to the FcγRIIa genotype (35,36). In breast cancer patients with metastatic disease receiving trastuzumab, the FcγRIIa V/V genotype compared to F...
carriers as well as FcγRIIa H/H genotype compared to R carriers was associated with a better clinical outcome. FcγRIIa V/V and/or FcγRIIa H/H identify the most favorable group responding to trastuzumab therapy (17). However, in a study where patients with metastatic CRC received cetuximab, FcγRIIa F carriers had a better survival than patients with the V/V genotype (18).

In follicular lymphoma and breast cancer, a better outcome was noted for FcγRIIa V/V and FcγRIIa H/H homozygote patients as compared to F/F and R/R, and no impact in heterozygous patients (V/F and H/R) (31,32). In our study, homozygosity for one allele in combination with heterozygosity for the corresponding allele constituted a favorable prognostic group. This might be explained by that one allele dose is sufficient for the Fcγ receptor to exert a sufficiently strong killing signal when the antibody once has bound to the receptor. This assumption is supported by experimental data showing that individuals who expressed at least one valine at FcγRIIa158 had higher levels of ADCC than F/F and that the number of FcγRIIa receptors was significantly higher among donors who expressed at least one valine (V/V and V/F) (37). However, this has to been demonstrated in further clinical trials.

The relation between FcγRIIa polymorphism and the outcome in MAb-treated patients is less studied. In follicular lymphoma and breast cancer, a better clinical response was noted for FcγRIIa131H/H patients as compared to the other FcγRIIa genotypes (17,19). Cheung et al (38) found that the FcγRIIa131R/R genotype was associated with a better clinical outcome in neuroblastoma patients treated with a murine anti-GD2 IgG3 MAb but Lin et al (39) found that FcγRIIaH131R polymorphism did not predict response to alemtuzumab (human IgG1) in CLL patients. The discrepancy between the studies may be due to different characteristics of the MAb. FcγRIIa receptors have various binding affinities for various classes of human IgG. IgG1 and IgG3 have a higher affinity than the other isotypes. R carriers have a strong affinity for mouse IgG1 as well as human IgG2 complexes (40). Edrecolomab, which is a mouse IgG2A, might have a strong affinity for R carriers as well as FcγRIIa H/H compared to R carriers with a superior clinical outcome in MAb-treated patients is our finding of a high ADCC activity of PBMC of V carriers. A higher ADCC activity of FcγRIIa158 V carriers has also been shown by others (Chan SL, et al, AACR Annual Meeting, abs. 2137, 2008) (17,37,42,43).

The absence of a relation between FcγR polymorphism and clinical efficacy in metastatic CRC patients probably is due to a low efficacy of the edrecolomab antibody in advanced disease.

Our model data may support that FcγR polymorphism might be of importance for the clinical effect of both passively and actively (vaccine) administered antibodies. FcγR polymorphism might be introduced as a biomarker for antibody-based treatment protocols as KRAS in antibody-treated CRC patients (44) to optimize the therapeutic strategy. Large prospective randomized trials are needed to establish the value of these biomarkers.

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