Published in: Alimentary Pharmacology (2004), vol. 19, pp. 511-519
Status: Postprint (Author's version)

Association between polymorphism in IgG Fc receptor IIIa coding gene and biological response to infliximab in Crohn's disease

E. LOUIS*, Z. EL GHOUL†, S. VERMEIRE‡, S. DALL'OZZO†. P. RUTGEERTS‡. G. PAINTAUD†. J. BELAICHE*. M. DE VOS§, A. VAN GOSSUM¶. J. -F. COLOMBEL** & H. WATIER†

** Department of Gastroenterology, CHU of Liège, Liège, Belgium; †Immuno-Pharmaco-Genetics of Therapeutic Antibodies' Research Group, UPRES-EA 3249, University and University Hospital of Tours. Tours, France; ‡Department of Gastroenterology, UZ Gasthuisberg, Leuven, Belgium; §Department of Gastroenterology, UZ Gent, Gent, Belgium; ¶Department of Gastroenterology, Erasme University Hospital, Brussels, Belgium; **Department of Gastroenterology, CHU Lille, Lille, France*

SUMMARY

Aim: To test the hypothesis of an association between polymorphism in *FCGR3A* (the gene coding for FcγRIIIa, which is expressed on macrophages and natural killer cells. is involved in antibody-dependent cell-mediated cytotoxicity and has recently been associated with a positive response to rituximab, a recombinant immunoglobulin G1 antibody used in non-Hodgkin's lymphomas) and response to infliximab in Crohn's disease.

Methods: FCGR3A-158 polymorphism was determined using an allele-specific polymerase chain reaction assay in 200 Crohn's disease patients who had received infliximab for either refractory luminal (n = 142) or fistulizing (n = 58) Crohn's disease. Clinical and biological responses (according to C-reactive protein levels) were assessed in 200 and 145 patients, respectively.

Results: There were 82.9% clinical responders in V/V patients vs. 72.7% in V/F and F/F patients (N.S.). Globally, the decrease in C-reactive protein was significantly higher in V/V patients than in F carriers (P = 0.0078). A biological response was observed in 100% of V/V patients, compared with 69.8% of F carriers (P = 0.0002: relative risk, 1.43: 95% confidence interval, 1.27-1.61). In the sub-group of patients with elevated C reactive protein before treatment. the multivariate analysis selected the use of immunosuppressive drugs and *FCGR3A* genotype as independent factors influencing the clinical response to infliximab (P = 0.003).

Conclusion: Crohn's disease patients with *FCGR 3A*-158 V/V genotype have a better biological and, possibly, clinical response to infliximab.

INTRODUCTION

Infliximab is a chimeric monoclonal inmmunoglobulin G1 (IgG1) antibody against tumour necrosis factor-α (TNF-α).[1] It is effective in refractory and listulizing Crohn's disease.[2, 3] In controlled trials. as well as in routine practice, the response rate to first treatment is around 75%. with half the responders showing a complete response and half a partial clinical response.[2-5] Certain demographic and clinical characteristics, including a young age,[6] colonic location of the disease,[6] co-treatment with immunosuppressive drugs[6, 7] and non-smoking,[7] have been associated with a positive response to the drug. Furthermore, some patients seem to be biologically predisposed to a non-response to infliximab, as manifested by the inefficacy of re-treatment in these patients.[2, 8] Pharmacogenetic hypotheses have been suggested to explain this phenomenon. Studies conducted on TNF-α and TNF receptor gene polymorphisms.[9-11] as well as on NOD2/ CARD15 gene mutations.[12- 13] have disclosed no strong association, although an association was suggested with a haplotype in the TNF-β (lymphotoxin-α) gene in a relatively small cohort.[8] and with the 5q31/IBD5 Crohn's disease risk haplotype in a larger cohort.[11] A better understanding of the mechanisms of action of infliximab in Crohn's disease may help in the identification of candidate genes. In addition to the neutralization of soluble TNF-α and blockade of its effect,[14] infliximab most probably induces the destruction of some key lamina propria mononuclear and polynuclear cells. 1[5- 16] The binding of infliximab to membrane TNF-α induces apoptosis in monocytes,[17] as well as cell lysis by complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity.[18] Antibody-dependent cell-mediated cytotoxicity is an important effector mechanism in the eradication of intracellular pathogens and tumour cells. It requires leucocyte receptors for the Fc portion of IgG (FcγR). whose function is to link the IgG-sensitized antigens to FcγR-bearing cytotoxic cells and to trigger cell activation. Such an antibody-dependent

cell-mediated cytotoxicity mechanism has also been suggested in the action of another recombinant IgG1 antibody, rituximab, used for the treatment of non-Hodgkin's lymphomas. [19-21] Recently, an important pharmacogenetic association has been shown with this antibody. A functionally significant polymorphism in *FCGR3A*.[22, 23] the gene coding for FcγRIIIa expressed on macrophages and natural killer cells, was associated with clinical and biological responses to rituximab in follicular non-Hodgkin's lymphomas.[24, 2S] This G → T potymorphism is located at nucleotide position +4985 (starting at the ATG initiation codon) in the sixth exon of *FCGR3A,* which is itself located on the long arm of chromosome 1 (lq23), within a cluster of five genes coding for the low-affinity FcγR.[26] The FcγRIIIa-158V (valine) allotype has a higher affinity for IgG1 than the FcγRIIIa-158F (phenylalanine) allotype, and natural killer cells from V/V subjects are more potent in antibody-dependent cell-mediated cytotoxicity at low antibody concentrations.[23] Non-Hodgkin's lymphoma patients with the V/V genotype showed a better clinical and biological response to rituximab, suggesting that, in these patients, the lysis of lymphomatous cells by antibody-dependent cell-mediated cytotoxicity was more effective, through better binding of the Fc portion of rituximab to natural killer cells.[24-. 25] Considering the cytolytic potential of infliximab, the aim of our work was to search for an association between the *FCGR3A*-158 polymorphism and biological and clinical responses to infliximab in Crohn's disease.

METHODS

Study population

Two hundred patients with Crohn's disease, included between November 1998 and August 2000 in an expanded access programme of infliximab in Belgium, were studied. All patients were treated with infliximab for the first time. To be included in the expanded access programme, patients had to be between 18 and 65 years of age, adopt adequate birth control measures, give informed consent and have one of the following specific inclusion criteria: (i) a single or multiple perianal or enterocutaneous draining fistula(e) as a complication of Crohn's disease, resistant to conventional treatment for at least 3 months; (ii) moderate to severely active Crohn's disease of at least 6 months' duration, with colitis, ileitis or ileo-colitis confirmed by radiography or endoscopy, refractory or dependent on oral steroid therapy (> 8 mg/day prednisone equivalent) and/or not responding to immunosuppressive agents (azathioprine, 6-mercaptopurine or methotrexate). Approval from the Ethics Committee was obtained in April 1998 and patients gave written informed consent for both infliximab treatment and pharmacogenetic ancillary studies. The demographic and clinical characteristics of the patients are given in Table 1. There were two sub-groups of patients: 142 patients with active luminal (non-fistulizing) disease and 58 patients with fistulizing disease. Luminal Crohn's disease patients were treated with a single infusion of infliximab (5 mg/kg). These patients were followed prospectively for 12 weeks, with physical examination, Crohn's disease activity index (CDAI) calculation and C-reactive protein (CRP) measurement (by routine procedure) at weeks 0, 4, S and 12. Patients with fistulizing disease were treated with three consecutive infusions (5 mg/kg) at weeks 0, 2 and 6. These patients were followed prospectively for 18 weeks, with physical examination (including Fistulous track drainage). CDAI calculation and CRP measurement (by routine procedure) at weeks 0, 2, 6. 10, 14 and 18.

Classification of response to infliximab

The response to infliximab was assessed on the basis of both clinical and biological evolution. Clinically, patients were classified as complete responders, partial responders or non-responders according to published controlled clinical trials.[2, 3] In non-fistulizing disease, patients were considered to be complete or partial responders at week 4 according to a decrease in CDAI below 150 or a decrease of 70 points from baseline, respectively. Furthermore, according to a recent National Institutes of Health Consensus Conference, we also looked at a partial response defined as a decrease of at least 100 points from baseline. In fistulizing disease, patients were considered to be complete or partial responders at week 10 according to complete fistula closure or a decrease of at least 50% in fistula drainage at two consecutive visits, respectively. Furthermore, the variation in CDAI between pre-treatment (week 0) and post-treatment (week 4 or 10) periods was calculated for all patients.

Biologically, patients were classified as complete, partial or non-responders at 4 weeks (non-fistulizing disease) or 10 weeks (fistulizing disease) on the basis of CRP evolution (measured by a routine procedure using immunoturbidimetry in local laboratories). This analysis was performed on a sub-group of 145 patients for whom CRP values were available before and after treatment and who showed an elevated CRP (greater than twice the upper limit of the normal range) before treatment. Patients were classified as complete or partial responders according to the normalization of CRP after treatment or a decrease of at least 25% from the baseline

level, respectively. Furthermore, for all patients, the variation of CRP (absolute and relative values) was calculated between pre-treatment (week 0) and post-treatment (week 4 or 10) periods.

Table 1. *Demographic, clinical and biological characteristics of the study population*

Mean Crohn's disease activity index (± s.d.)	259 (± 116)
Mean C-reactive protein (normalized) (± s.d.)	11.2 (± 14.6)
NOD2/CARD15 genotype	
Wild type (%)	137 (68.5)
Heterozygotes (%|	50 (25.0)
Homozygotes and compound	13 (6.5)
heterozygotes (%)	
Female/male (%)	122/78 (61.0/39.0)
Fistulizing/refractory (%)	58/142 (29.0/71.0)
Median age (years) (interquartile range)	34 (25-47)
Disease location	
Ileal (%)	39 (19.5)
Colonic (%)	69 (34.5)
Ileo-colonic (%)	81 (40.5)
Anal involvement (%)	77 (38.5)
Upper gastrointestinal involvement (%)	11 (5.5)
Concomitant treatment	
5-Aminosalicylates (%)	94 (47.0)
Corticosteroids {%)	86 (43.0)
Azathioprine/6-mereaptopurine (%)	111 (55.5)
Methotrexate (%)	15 (7.5)
Total immunosuppressives (%)	126 (63.0)
Smoking (%)	68 (34.0)

FCGR3A-158V/F genotyping

Genomic DNA was isolated using phenol/chloroform extraction, as described previously,[12] and stored at -80 °C until use. Allele-specific polymerase chain reactions (PCRs) were performed with primers partially based on those previously designed by Leppers-van de Straat *et al.*[27] and recently described,[28] The forward primer (5'-TCCAAAAGCCACACTCAAAGTC-3') includes a 3' penultimate mismatch (underlined) lo completely avoid *FCGR3B* amplification and to allow *FCGR3A*-specilic amplification. The V allele-specific (5'-GGGGGGCCCCGGGGGTGATGTTCACAGTCTGAGAAGA CACATTTTTACTCCCTAC-3') and the F allele-specific (5'-AGACACATTTTTACTCCCATA-3') (Eurobio. Les Ulis, France) reverse primers also included new 3' mismatches (underlined) to enhance allele specificity. Moreover, the V allele reverse primer was 5' elongated (italic characters) to allow single-tube amplification and allele discrimination after electrophoresis. PCRs were carried out in a final volume of 25 μL consisting of *Taq* polymerase buffer (670 mM Tris-HCl pH 8.8, 160 mM $(NH_4)_2SO_4$. 0.1% Tween 20), 2 mM $MgCl_2$, 400 μM dNTP (MBI Fermentas, Vilnius, Lithuania), 20 pmol of the common forward primer, 14.5 pmol of the V allele-specific reverse primer and 30 pmol of the F allele-specific reverse primer (all three synthesized by Genset SA, Paris, France), 1 unit of *Taq* DNA polymerase (Eurobio) and 2 μL genomic DNA. PCR was set up in a GeneAmp PCR system 2400 (Perkin Elmer France SA, Saint Quentin en Yvelines, France) programmed for an initial denaturation step of 5 min at 95 °C, followed by 35 cycles at 94 °C for 20 s, hybridization at 58 °C for 20 s and elongation at 72 °C for 20 s. PCR products were then analysed by electrophoresis in 8% polyacrylamide gels run in TBE buffer (90 mM Tris-HCl, 90 mM boric acid, 2.5 mM ethylenediaminetetra-acetic acid) (Eurobio). After staining with ethidium bromide (Euro-bio), gels were visualized using ultraviolet transillumination (Gel Doc 1000 system. Bio-Rad, Hercules. CA, USA) and images were captured on a Kodak Digital Science Image (Kodak, Rochester, NY, USA). The F and V allele amplification products appeared as bands of 70 bp and 102 bp. respectively.

Statistical analyses

CRP values were normalized and expressed as a multiple of the upper limit of the normal range determined in each laboratory (95% reference interval, as recommended by the International Federation of Clinical Chemistry[29]). Response rates according to various *FCGR3A*-158V/F genotypes were compared using the chi-

squared test. Comparisons were performed between the three genotypes (V/V, V/F, F/F) and also between V carriers (V/V, V/F) and non-carriers (F/F), as well as between F carriers (F/F. V/F) and non-carriers (V/V). Variations in CDAI between pre- and post-treatment periods (δCDAI) and variations in CRP between pre- and post-treatment periods (δCRP) were compared by *t*-test or Mann-Whitney test as required.

Multivariate analyses by stepwise logistic regression were performed. An initial analysis was performed, with the clinical response as the dependent variable, on a sub-group of 189 patients for whom all the demographic, clinical and biological data were available. A second analysis was performed, with the biological response as the dependent variable, on a sub-group of 145 patients who showed an elevated CRP before treatment. A third multivariate analysis, with the clinical response as the dependent variable, was also performed on this sub-group of 145 patients.

P values of < 0.05 were considered to be significant.

RESULTS

Clinical and biological responses to infliximab

Clinically, there were 106 (53.0%). 43 (21.5%) and 51 (25.5%) complete, partial and non-responders, respectively, in the study. In patients with non-fistulizing disease, there were 79 (55.6%). 30 (21.1%) and 33 (23.3%) complete, partial and non-responders. respectively. In patients with fistulizing disease, there were 27 (46.6%), 13 (22.4%) and 18 (31.0%) complete, partial and non-responders. respectively. Biologically, as determined by the decrease in serum CRP, there were 52 (35.9%), 58 (40.0%) and 35 (24.1 %) complete, partial and non-responders, respectively.

Clinical and biological responses to infliximab according to FCGR3A-158V/F genotypes

There were 35 (17.5%), 100 (50.0%) and 65 (32.5%) *PCGR3A*-158V/V, V/F and F/F patients, respectively. The frequencies of clinical complete, partial and non-responders, according to the *PCGR3A* genotype, are shown in Table 2. There was no significant difference when comparing the various genotypes or V and F carriers and non-carriers. Even when looking at partial responses delined using more stringent criteria (decrease of at least 100 points in CDAI), there was still no significant difference between the groups (80.0% responders in V/V patients vs. 63.0% responders in V/F and F/F patients: $P = 0.11$). The median δCDAI was -161 (range, +65 to -427) in V/V homozygous patients vs. -116 (range, +135 to -349) in the F carrier group ($P = 0.5$). The median duration of response was 14 weeks (range, 6-104 weeks) in the V/V genotype vs. 12 weeks (range, 1-192 weeks) in the V/F and F/F genotypes ($P = 0.9$).

The frequencies of biological complete, partial and non-responders, according to the *FCGR3A* genotype, are shown in Table 3. Overall, there was a significant difference between genotypes ($P = 0.01$). The best response was observed in V/V homozygotes and the worst response was found in F/F homozygotes. The most striking difference was observed when looking at the response rate (complete or partial) among V/V homozygotes when compared with other genotypes (Figure 1). The median δCRP (relative value) was -80.1% (range. -31.0% to -94.8%) in V/V homozygotes vs. - 63.2% (range. + 1100% to -98.1%) in V/F and F/F patients together ($P = 0.0078$) (Figure 2). The median δCRP (absolute value, normalized) was -8.0 (range, -1.0 to -119.0) in V/V patients vs. -6.6 (range, +107.0 to -58) in the F carrier group ($P = 0.05$).

The frequencies of clinical complete, partial and non-responders, according to the *FCGR3A* genotype, in the sub-group of 145 patients (including 1.13 luminal and 32 fistulizing Crohn's disease) with elevated CRP before treatment, are shown in Figure 3. Globally, there was no significant difference but, when comparing only the frequencies of non-responders with complete and partial responders grouped together, there was a trend towards a higher response rate in V/V patients ($P = 0.15$), relative risk. 1.19: 95% CI. 0.99-1.43.

Table 2. *Clinical response to infliximab according to PCGR3A genotype (%)*

Genotype	Complete response	Partial response	Non-responders
All patients (*n* = 200)			
V/V (*n* = 35)	21 (60.0)	8 (22.9)	6 (17.1)
V/F (*n* = 100)	51 (51.0)	22 (22.0)	27 (27.0)
F/F (*n*= 65)	34 (52.3)	13 (20.0)	18 *(27.7)*
Non-fistulizing disease (*n* = 142)			
V/V (*n = 12)*	13 (59,1)	6 (27.3)	3 (13.6)
V/P (*n*= 74)	44 (59.5)	14 (18.9)	16 (21.6)
F/F (*n* = 46)	22 (47.8)	10 (21.7)	14 (30.5)
Fistulizing disease (*n* = 58)			
V/V (*n* = 13)	8 (61.5)	2 (15.4)	3 (23.1)
V/F (*n* = 26)	7 (26.9)	8 (30.8)	11 (42.3)
F/F (*n* = 19)	12 (63.2)	3 (15.8)	4 (21.0)

Table 3. *Biological response to infliximab according to PCGR3A genotype (%)*

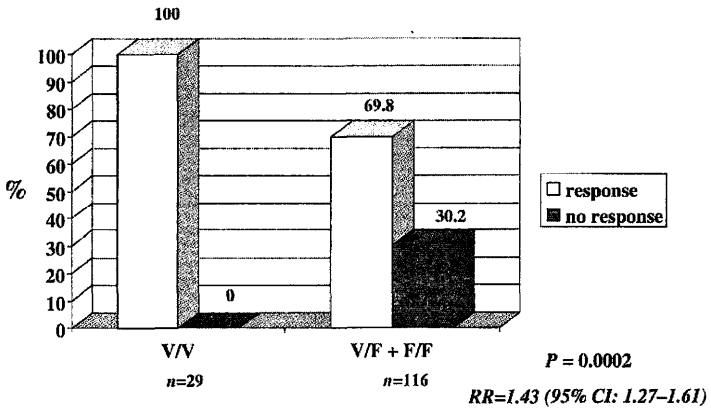
Genotype	Complete response	Partial response	Non-responders
All patients* (*n* = 145)			
V/V (*n* = 29)	13 (44.8)	16 (55.2)	0 (0)
V/F (*n*= 71)	27 (38.0)	24 (33.8)	20 (28.2)
F/F (*n* = 45)	12 (26.7)	18 (40.0)	15(33.3)

Complete response, normalization of C-reactive protein 4 weeks after treatment.
Partial response, decrease in C-reactive protein of at least 25%
4 weeks after treatment.
P = 0.01 (3 × 3 contingency table).
** Including 113 luminal and 32 fistulizing disease.*

Multivariate analyses

The logistic regression with clinical response as the dependent variable, on the whole group of patients for whom all clinical, demographic and biological data were available before treatment (*n* = 189), identified the use of immunosuppressive drugs and an elevated CRP level (greater than twice the upper limit of the normal range) as factors predictive of response (*P* = 0.003). The logistic regression with biological response as the dependent variable, on the sub-group of 145 patients with an elevated CRP before treatment, could not be performed because the *FCGR3A* genotype gave a complete separation of the patients (100% of responders amongst the V/V homozygotes). The logistic regression with clinical response as the dependent variable, on the sub-group of 145 patients with elevated CRP before treatment, selected the use of immunosuppressive drugs and the *FCGR3A*-158V/V genotype as factors predictive of response (*P* = 0.003).

Figure 1. *Biological response to infliximab in patients with elevated C-reactive protein (CRP) (greater than twice the upper limit of the normal range) before treatment according to FCGR3A-158 genotype. Complete and partial biological responses were defined as a normalization of CRP or a decrease of at least 25%. respectively, 4 weeks after treatment. CI. confidence interval: RR. relative risk.*



Published in: Alimentary Pharmacology (2004), vol. 19, pp. 511-519
Status: Postprint (Author's version)

DISCUSSION

Our results show a significant association between a biological response to infliximab, assessed by the variation in CRP level, and a single nucleotide polymorphism in the *FCGR3A* gene, which codes for a receptor for the Fc portion of IgG (FcγR) on natural killer cells and macrophages. There was also a trend towards an association between this polymorphism and a clinical response to infliximab.

Several arguments may explain why the *FCGR3A* effect was more prominent when a biological marker was considered (the CRP serum concentration), related to mucosal immuno-inflammatory processes, rather than the clinical response (adopting a CDAI decrease of 70 or 100 points). First, a clinical evaluation of the response to treatment is sometimes difficult in Crohn's disease and the correlation between clinical and biological parameters is poor.[30, 31] Second, the symptoms and clinical activity in Crohn's disease may result from a wide range of factors, and not only inflammation which is targeted by infliximab. Finally, certain non-responses to infliximab may be related to an absence of significant inflammation before treatment,[9] such as in clinical forms of Crohn's disease characterized by a predominance of irritable bowel syndrome, or post-surgical symptoms for which infliximab has no impact and the *FCGR3A* gene polymorphism no influence. Indeed, when the sub-group of patients with elevated CRP before treatment was selected, a trend towards an association between the *FCGR3A* gene polymorphism and a clinical response to infliximab was observed.

Similar to rituximab used in the treatment of follicular non-Hodgkin's lymphomas.[24, 25] *FCGR3A*-158V/V homozygous patients developed a better response to infliximab, whereas sub-optimal responses were observed in F/F homozygotes. According to the association suggested by the clinical response to rituximab.[24, 25] V homozygosity confers a functional advantage, whereas the carriage of a single V allele in heterozygotes does not seem to be sufficient for a better response than in F homozygotes. This is the reason why the majority of the analyses were performed by grouping V/F and F/F patients together. The most striking difference concerned the proportion of biological non-responses: none in V/V patients vs. 30.2% in F carriers. This difference was also noted for the clinical response (although not statistically significant): in patients with elevated CRP before treatment, only 13.8% of V/V patients did not clinically respond vs. 27.6% of F carriers; in the whole group of patients, 17.1% of the V/V patients showed no response vs. 27.3% of F carriers. An analysis of a larger number of patients will be required to reach a more striking statistical significance concerning the association between the *FCGR3A* polymorphism and the clinical response to infliximab. According to our data, however, for a given patient, the predictive value of the *FCGR3A* genotype on the clinical response to infliximab in Crohn's disease is low.

Figure 2. *Variation in the relative value of C-reactive protein (CRP) 4 weeks after infliximab treatment (compared with baseline) according to FCGR.3A-158 genotype. (a) Relative CRP variation in F carriers (V/F and F/F genotypes): the median variation was - 63.2% (range, + 1100% to - 98.1%). (b) Relative CRP variation in V/V patiente: the median variation was -80.1% (range, -31.0% to -94.8%).*

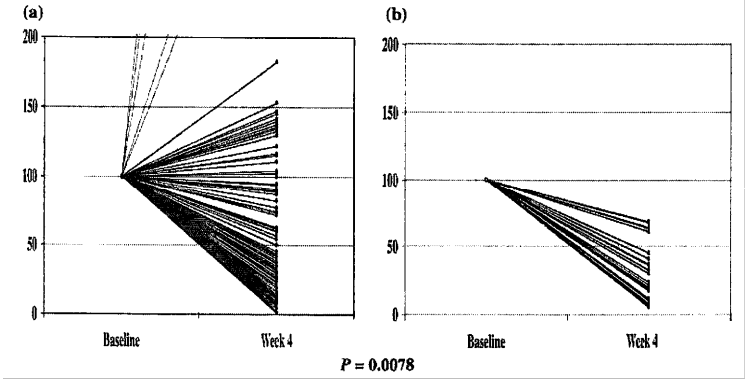
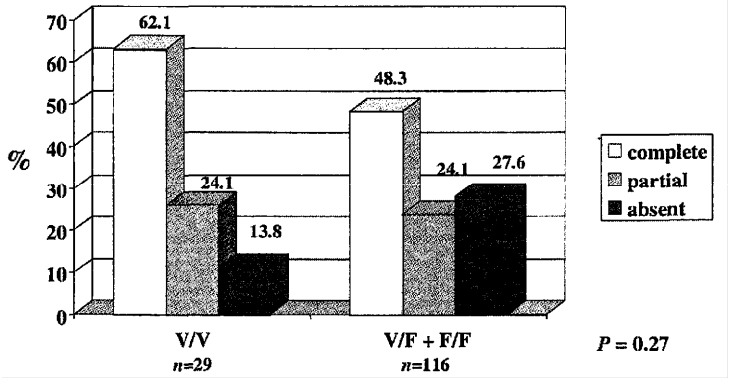


Figure 3. *Clinical response to infliximab according to FCGR3A-158 genotype in a sub-group of patients with elevated C-reactive protein (CRP) (greater than twice the upper limit of the normal range) before treatment. For luminal Crohn's disease (n = 113), complete and partial responders were defined by a Crohn's disease activity index (CDAI) below 150 or a decrease of at least 70 points 4 weeks after treatment, respectively. For fistulizing Crohn's disease (n= 32), patients were considered as complete or partial responders at week 10 according to complete fistula closure or a decrease of at least 50% in fistula drainage at two consecutive visits, respectively.*



The response to infliximab treatment is probably a complex phenomenon influenced by several parameters. In this model, the individual impact of each particular factor may not be very strong. So far. various clinical and demographic parameters, including the use of immunosuppressive drugs[6, 7] a young age.[6] colonic location,[6] non-smoking[7] and elevated CRP.[9] have been associated with a positive clinical response to infliximab, but no relevant pharmacogenetic association has been established. We studied all of these clinical and demographic parameters in a multivariate analysis with clinical response as the dependent variable. This analysis identified the use of immunosuppressive drugs and the presence of an elevated CRP level as factors predictive of a response to infliximab. In the sub-group of patients with an elevated CRP before treatment, the multivariate analysis identified the use of immunosuppressive drugs and the *FCGR3A* polymorphism as predictive factors. These results strengthen the case for a significant influence of the *FCGR3A* genotype on the response to infliximab, independent of other factors previously identified.[7]

The *FCGR3A* gene studied encodes FcγRIIIa. a receptor for the Fc portion of IgG expressed on macrophages and natural killer cells and involved in antibody-dependent cell-mediated cytotoxicity. This mechanism has been identified *in vitro* using infliximab and cells bearing transmembrane TNF-α.[18] The relative importance of antibody-dependent cell-mediated cytotoxicity in the therapeutic efficacy of infliximab *in vivo,* in addition to other mechanisms described in *vitro,* such as complement activation,[18] apoptosis induction[17] or TNF-α neutralization,[32] is not known. However, the superiority of infliximab over etanercept in the treatment of Crohn's disease[33] may be linked to a different ability to bind transmembrane TNF-α and. consequently, to induce the killing of activated TNF-α-positive mononuclear cells.[34] Indeed, although the capacity to bind soluble TNF-α was similar with the two drugs, only infliximab was able to induce apoptosis of activated lymphocytes *in vitro.*[34] In this *in vitro* model, neither complement-mediated nor cell-mediated lymphocyte lysis seemed to be involved. In another model, however, infliximab and etanercept also showed a different ability to mediate complement-dependent killing of TNF-α-expressing cells.[35] *In vivo,* as demonstrated in some *in vitro* experiments.[18, 36] cell-mediated lysis, as a consequence of infliximab binding to transmembrane TNF-α, may further contribute to the efficacy of infliximab. The functionally significant *FCGR3A-15S* polymorphism may thus have an impact on infliximab efficacy by influencing its ability to kill mucosal mononuclear cells through antibody-dependent cell-mediated cytotoxicity, and thus may influence the rate of sustained response measured 4 weeks after treatment. This suggests that antibody-dependent cell-mediated cytotoxicity is one of the mechanisms involved in the action of infliximab, and that V/V patients are more likely than F carriers to have at least a biological response through this mechanism. However, further genetic studies (excluding linkage disequilibrium) and *in vitro* experiments will be required to demonstrate these hypotheses.

In conclusion, our results show, for the first time, a relevant pharmacogenetic association for infliximab in Crohn's disease. They suggest a role for FcγR and probably antibody-dependent cell-mediated cytotoxicity amongst the mechanisms of action of infliximab in Crohn's disease. Finally, they also emphasize the need for a biological, and not only clinical, assessment of response in Crohn's disease when studying new drugs, especially in pharmacogenetic investigations.

ACKNOWLEDGEMENTS

This work was supported by the Fondation Langlois. E. Louis is a Research Associate for the FNRS Belgium. S. Vermeire is a Research Fellow for the NFWO Belgium. S. Dall'Ozzo was supported by a grant from CANCEN and the Conseil Régional du Centre.

An operating grant from the broad medical research program (The Eli and Edythe Broad Foundation) was obtained.

REFERENCES

1 Knight DM. Trinh H. Le J, *et al.* Construction and initial characterization of a human-mouse chimeric antibody. Mol Immunol 1993: 30; 1443-53.

2 Targan S. Hanauer S, van Deventer SJH, *et al.* A short term study of chimeric antihody cA2 to tumor necrosis factor alpha for Crohn's disease. N Engl J Med 1997: 337: 1029-35.

3 Present DH, Rutgeerts P. Targan S. *et al.* Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med 1999: 340: 1398-405.

4 Cohen RD, Tsang JF. Hanauer SB. Infliximab in Crohn's disease: first anniversary clinical experience. Am J Gastroenterol 2000: 95: 3469-77.

5 Farrell RJ. Shah SA. Lodhavia PJ. *et al.* Clinical experience with infliximab therapy in 100 patients with Crohn's disease. Am J Gastroenterol 2000; 95: 3490-7.

6 Vermeire S, Louis E, Carbonez A, *el al.* Demographic and clinical parameters influencing the short-term outcome of anti-tumor necrosis factor (infliximab) treatment in Crohn's disease. Am J Gastroenterol 2002: 97: 2357-63.

7 Parsi MA, Achkar JP. Richardson S. *et al.* Predictors of response to infliximab in patients with Crohn's disease. Gastroenterology 2002: 123: 707-13.

8 Taylor KD, Plevy SE, Yang II. *et al. A*nca pattern and LTA haplotype relationship to clinical responses to anti-TNF antibody treatment in Crohn's disease. Gastroenterology 2001; 120: 1347-55.

9 Louis E. Vermeire S, Rutgeerts P, *el al.*. A positive response to infliximab in Crohn's disease: association with a higher systemic inflammation before treatment but not with -308 TNF gene polymorphism. Scand J Gastroenterol 2002: 37: 818-24.

10 Mascheretti S, Hampe J. Kuhbacher T, *et al.* Pharmacogenetic investigation of the TNF/TNF-receptor system in patients with chronic active Crohn's disease treated with infliximab. Phar-macogenomics J 2002; 2: 127-36.

11 Dc Silva A, Vermeire S. Ahmad T, *et al.* Pharmacogenetics of infliximab in Crohn's disease: the 5q31/IBD5 risk haplotype predicts response. Gastroenterology 2002: 122: M1423.

12 Vermeire S. Louis E. Rutgeerts P, *et al.* NOD2/CARD15 does not influence response to infliximab in Crohn's disease. Gastroenterology 2002; 123: 106-11.

13 Mascheretti S. Hampe J, Croucher PJ, *et al.* Response to infliximab treatment in Crohn's disease is not associated with mutations in the CARD15 (NOD2) gene: an analysis in 534 patients from two multicenter, prospective GCP-level trials. Pharmacogenetics 2002; 12: 509-15.

14 Lorenz HM. Antoni C. Valerius T, *et al.* In vivo blockade of TNF-alpha by intravenous infusion of a chimeric monoclonal TNF-alpha antibody in patients with rheumatoid arthritis. Short-term cellular and molecular effects. J Immunol 1996: 156: 1646-53.

15 Geboes K, Dalle I, Baert F, D'Haens G. Cornillie F, Rutgeerts P. Mucosal distribution of infliximab in patients with Crohn's disease. Gastroenterology 2001: 120: A1664.

16 Baert FJ, D'Haens G, Peeters GR. *et al.* Tumor necrosis factor alpha antibody (infliximab) therapy profoundly down-rcgu-latcs the inflammation in Crohn's ileo-colitis. Gastroenterology 1999; 116: 22-8.

17 Lügering A. Schmidt M. Lugering N. Pauels AG, Domschke W. Kucharzik T. Infliximab induces apoptosls in monocytes from patients with chronic active Crohn's disease by using a ca-spasc-dependent pathway. Gastroenterology 2001; 121: 1145-57.

18 Scallon BJ, Moore MA, Trinh II, Knight DM. Ghrayeb J. Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions, Cytokine 1995; 7: 251-9.

19 Reff ME. Carner K. Chambers KS. *et al.* Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994: S3: 435-45.

20 Flieger D, Renoth S, Beier I, Sauerbruch T, Schmidt-Wolf I. Mechanism of cytotoxicity induced by chimeric mouse human monoclonal antibody IDEC-C2B8 in CD20-expresslng lymphoma cell lines. Cell Immunol 2000: 204: 55-63.

21 Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med 2000; 6: 443-6.

22 Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de Haas M. FcγRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell FcγRIIIa, independently of the FcγRIIIa-48L/R/H phenotypc. Blood 1997; 90: 1109-14.

23 Wu J, Edberg JC. Redecha PB. *et al.* A novel polymorphism of FcγRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. J Clin Invest 1997: 1000: 1059-70.

24 Cartron G, Dacheux L. Salles G. *et al.* Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. Blood 2002; 99: 754-8.

25 Weng WK, Levy R. Two immunoglobulin G Fc receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 2003; 21: 3940-7.

26 Su K, Wu J, Edberg JC. McKenzie SK, Kimberly RP Genomic organization of classical human low-affinity Fcγ receptor genes. Genes Immunity 2002: 3(Suppl. 1): S51-6.

27 Leppers-van de Straat FG, van der Pol WL. Jansen MD, *et al. A* novel PCR-based method for direct Fc gamma receptor Ilia (CD16) ailotyping. J Immunol Methods 2000: 242: 127-32.

28 DalΓOsαo S. Andres C. Bardos P, Watier H. Thibault G. Rapid single-step *FCGR3A* genotyping based on SYIÎR Green I fluorescence in real-time multiplex allele-specilic PCR. J Immunol Methods 2003: 277: 185-92.

29 Solberg FIR. Approved recommendation on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J Clin Chem Clin Biochem 1987: 25: 645-56.

30 Cellier C. Sahmoud T. Froguel E. *et al* Correlations between clinical activity, endoscopic severity, and biological parameters in colonic or ilcocolonic Crohn's disease. A prospective multicentre study of 121 cases. Gut 1994; 35: 231-5.

31 D'Haens G. Van Dcvcnter S. Van Hogezand R, *etal.* Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: a European multicenter trial. Gastroenterology 1999; 116: 1029-34.

32 Siegel SA. Sheay 1)J, Nakada MT, *et al.* The mouse/human chimeric monoclonal antibody cA2 neutralizes TNF in vitro and protects transgenic mice from cachexia and TNF lethality in vivo. Cytokine 1995; 7: 15-25.

33 Sandborn WJ, Hanauer SB. Kate S. *et al.* Etanercept for active Crohn's disease: a randomised, double-blind, placebo-controlled trial. Gastroenterology 2001: 121: 1088-94.

34 Van den Brande J, Braat II, van den Brink G. *et al* Infliximab but not etanercept induces apoptosis in lamina propria T-lymphoeytes from patients with Crohn's disease. Gastroenterology 2003; 124: 1774-85.

35 Barone D, Krantz D. Maiori K. Moler K. Comparative analysis of the ability of etanercept and infliximab to lyse TNF-expressing cells in a complement-dependent fashion. Arthritis Rheum 1999; 42(Suppl·): S90.

36 Shen C, Colpaert S. Maerten P. *et al.* Infliximab induces death of human monocytes in vitro and in the THP-1-SCID-mouse model. Clin Immunol Suppl 2003; 1: S142.