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A Positive Response to Infliximab in Crohn Disease: Association with a Higher Systemic Inflammation Before Treatment But Not With -308 TNF Gene Polymorphism

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Abstract

Background: Two-thirds to three-fourths of patients with either refractory luminal or fistulizing Crohn disease respond to infliximab treatment. The ability or inability to respond seems to persist over time. Biological characteristics and/or genetic background can influence the response to treatment. The aim was to assess the value of C-reactive protein and TNF-α serum levels before treatment as well as the TNF -308 gene polymorphism in the prediction of response to infliximab treatment in Crohn disease. Methods: Two-hundred-and-twenty-six Crohn disease patients treated in the setting of an expanded access programme to infliximab in Belgium were studied. There were 136 refractory luminal diseases and 90 refractory fistulizing diseases. Luminal diseases were treated with one single infusion; fistulizing diseases with three infusions at weeks 0,2 and 6. A clinical response to treatment was defined as either a Crohn disease activity index <150 (complete) or a drop of 70 points (partial) at week 4, for luminal disease, and as either complete fistula healing (complete) or a decrease of at least 50% of the number of draining fistulas on two consecutive visits between weeks 0 and 18, for fistulizing disease. CRP and serum TNF-α levels were measured at week 0 before treatment and were compared between responders and non-responders. Patients were genotyped for the -308 TNF gene polymorphism, and allelic as well as genotype frequencies were compared between responders and non-responders. Results: There were 73.2% responders (46.4% complete and 26.8% partial) and 26.8% non-responders. Response rates were similar in luminal and fistulizing diseases. CRP level before treatment was significantly higher in responders than in non-responders (16.8 mg/l (5-160) versus 9.6mg/l (5-143); $P = 0.02$). Furthermore, response rate was significantly higher in patients with elevated CRP (>5 mg/l) than in patients with a normal CRP value (<5mg/l) before treatment (76% versus 46%; $P = 0.004$; OR: 0.26 (0.11-0.63)). Allelic and genotype frequencies for -308 TNF gene polymorphism were not significantly different between responders and non-responders - with the exception of a slightly higher TNF2 frequency in non-responders in luminal disease (22.1 % versus 11.6%; $P = 0.04$). However, this was not associated with a significant difference in genotype frequencies. Conclusion: A positive clinical response to infliximab was associated with a higher CRP level before treatment in our population of Crohn disease patients, but there was no relevant association with -308 TNF gene polymorphism. We therefore suggest that CRP level may help to identify better candidates for infliximab treatment.

Key words : Crohn disease ; genetics ; infliximab ; tumour necrosis factor

Infliximab has shown a dramatic efficacy in both refractory luminal and fistulizing Crohn disease (1,2). What is impressive is not the percentage of response to this treatment, but rather the way the patients respond: quickly, with a parallel improvement in their quality of life (1,2) and with a scaring of the lesions at endoscopy (3,4) and histology (4,5). Such a dramatic response is observed in one-third of patients, while another third may present a partial response (1,2). Obviously, because of the cost and the possible side effects of this new biological therapy, prognostic factors for the response to infliximab would be most welcome. Furthermore, the clinical impression, also confirmed by biological and histological parameters, that we can define responding and non-responding patients to the drug is another argument for the need to search for prognostic markers.

The clinical trials searching for prognostic factors such as the epidemiological and clinical characteristics of patients have not yet disclosed any relevant marker (1,2). More specific research should be based on the mechanism of action of infliximab. So far this is only partly understood (6,7). Infliximab blocks soluble TNF-α

and thus TNF-driven inflammation. The central role of TNF-α in the inflammatory process characterizing Crohn disease has been well documented (8-12). One could thus speculate that the response to infliximab may depend on the intensity of this TNF-driven inflammation, an absence of response being due either to an excess of inflammation insufficiently controlled by infliximab or to another type of inflammation not dependent on TNF-α. In this perspective, CRP levels can be considered as a good sign of systemic inflammation in Crohn disease (14-16), and systemic TNF-α levels may indicate the implication of TNF-α in the inflammatory process. Another important factor to take into account is that ability/inability to respond to infliximab is something that seems to persist over time (17). Therefore a genetic predisposition may well be involved in this ability to respond. Several polymorphisms have been described in the TNF gene (18-21). Among them a single base pair substitution at position -308 in the promoter region of the gene has been the most studied (19). The less frequent allele TNF2 of this polymorphism has been associated with an increased capacity of TNF transcription and production in several in vitro (22,23) and ex vivo models (24) and with diseases characterized by an increased production of TNF-α (25-29). Recently, we demonstrated that the TNF2 allele was associated with more severe, either steroid-dependent or fistulizing Crohn disease (30). The aim of our work was to assess the possible association between serum TNF-α and CRP levels before treatment, as well as -308 TNF genotype and response to infliximab.

Table I: *Patient characteristics at the time of infliximab treatment*

	Luminal disease	**Fistulizing disease**
No. of patients	136	90
% Male	40.4	36.7
Age (mean ± *s* years) (range)	35.9 (±11.6) (17-74)	37.8 (±10.9) (16-66)
Duration of Crohn disease (mean ± *s* years) (range)	8.5 (±6.6) (1-30)	8.6 (±5.7) (1-30)
Location of the disease (%) (ileum/ileocolon/colon)	17.6/52.2/30.1	17.8/37.8/44.4
CDAI (mean = *s*) (range)	303.8 (±100.4) (158-609)	195.7 (±113.1) (31-526)
% on steroids	54.4	36.7
% on 5-ASA or salazopyrine	40.4	53.3
% on 6-mercuptopurine or azalhioprine	47.8	55.6
% on methotrexate	8.8	1.1
% on antibiotics	5.9	32.2
% on cyclosporine	0	1.1
% on FK 506	0.7	0

s = standard deviation.

Patients and Methods

Crohn disease patients

Two-hundred-and-twenty-six patients with Crohn disease were studied, all of them consecutively included in an expanded access programme of infliximab launched by Schering Plough in Belgium and Luxembourg. The diagnosis of Crohn disease was based on clinical, radiological and histological data in accordance with standard criteria. To be included in the expanded access programme the 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: (1) to have single or multiple enterocutaneous draining fistula(e) as a complication of Crohn disease (including external and internal) resistant to conventional treatment for at least 3 months, (2) to have moderate to severe active Crohn disease of at least 6 months' duration, with colitis, ileitis or ileocolitis confirmed by radiography or endoscopy and refractory or dependent on oral corticosteroid therapy (>8 mg/day prednisone equivalent). These patients had to have been already treated, intolerant or non-responding to more classical immunosuppressants (azathioprine, 6-mercaptopurine or methotrexate).

The clinical characteristics of the patients are given in Table I. There were two subgroups of patients: 136 patients with active luminal (non-fistulizing) disease and 90 patients with fistulizing disease. Luminal Crohn disease patients were treated with a single infusion of infliximab (5 mg/kg). Before treatment, a blood sample and a serum sample were harvested for DNA extraction and serum TNF-α measurement, respectively. These patients were prospectively followed for 12 weeks with a physical examination, Crohn disease activity index

(31) (CDAI) calculation, CRP measurement at weeks 0,4,8 and 12. According to Targan et al. (I), they were considered as complete responders or partial responders in the case of a decrease of CDAI below 150 or a decrease of 70 points in CDAI, respectively, after 4 weeks. Patients with fistulizing disease were treated with three consecutive infusions (5 mg/kg) at weeks 0, 2 and 6. Before treatment blood and serum samples were harvested for DNA extraction and serum TNF-α measurement, respectively. These patients were prospectively followed up for 18 weeks with physical examination, CDAI calculation and CRP measurement at weeks 0, 2,6, 10, 14 and 18. According to Present et al. (2), they were considered as complete responders and partial responders in the case of complete fistulas healing and a decrease in 50% of the number of draining fistulas at two consecutive visits, respectively. Ethical approval for this genetic study was given by the Ethics Committee of the Faculty of Medicine of Liege in March 1995. All the patients gave their informed consent specifically for the genetic study.

Table II: *CRP levels (mg/l) expressed as median and range before infliximab treatment according to response to treatment*

	Responders	**Non-responders**
Whole group of patients (109 responders/44 non-responders)	16.8 (5-160)	9.6 (5-143.5)*
Luminal Crohn disease (60 responders/25 non-responders)	16.5 (5-160)	11 (5-143.5)
Fistulizing Crohn disease (49 responders/19 non-responders)	16.8 (5-134)	8.1 (5-39.5)

* *P = 0.02.*

Controls

One-hundred-and-twenty-eight unrelated healthy European Caucasians who served as controls for the genotyping were either hospital workers or prospective blood donors. None had a personal or familial history of inflammatory bowel disease.

DNA extraction

Genomic DNA was extracted from 10 ml venous blood using a modified salting out technique (32) and re-suspended in sterile distilled water at a final concentration of 0.1-1.0 µg/µl, before use.

TNF -308 SBP substitution

The substitution was studied either by a polymerase chain reaction involving primers specific for each allele of the G to A polymorphism at residue -308, as previously described (33), or by PCR followed by NCO1 digestion. After amplification or digestion, DNA samples were eleclrophoresced in 1% (allele specific PCR) or 2% (PCR and NCO1 digestion) agarose gels containing ethidium bromide, and visualized under ultraviolet light.

Measurement of TNF-α serum levels

TNF-α serum level was measured on the serum samples harvested at week 0 before infliximab treatment. It was measured in duplicate using commercial EASIA (Biosources, Europe) in accordance with the manufacturer's indications. The level of TNF-α detection was 10 pg/ml.

Statistics

For CRP and TNF-α serum levels, the values below the detection threshold were all put at the threshold level (5 mg/l for CRP and 10 pg/ml for TNF-α). CRP and TNF-α serum levels were expressed as median and range. Comparison between CRP levels before and after infliximab treatment was made using a paired non-parametric test (Wilcoxon signed rank test).

CRP and TNF-α levels before treatment were compared in responders and non-responders using the Mann-Whitney Utest. Furthermore, an optimal discriminant value between responders and non-responders was sought by logistic procedure and ROC curve. The proportions of responders in groups according to this optimal discriminant value were compared using a Fisher exact test, with calculation of the odds ratio.

Allelic and genotype frequencies for -308 TNF gene polymorphism were compared between responders and non-responders using the Fisher exact test, with calculation of the odds ratio. Level of significance for all these tests was $P < 0.05$.

Results

Clinical and biological response to infliximab

Overall, there were 220/226 patients evaluable for the response to infliximab treatment: 102 were complete responders (46.4%), 59 were partial responders (26.8%) and 59 were non-responders (26.8%). Among the luminal Crohn disease patients, there were 57/133 (42.9%) complete responders, 42/133 (31.6%) partial responders and 34/133 (25.6%) non-responders. Among the fistulizing Crohn disease patients, there were 45/87 (51.7%) complete responders, 17/87 (19.5%) partial responders and 25/87 (28.7%) non-responders.

The clinical response was associated with a significant decrease in CRP in the responder group (16.5 mg/l (5-160) at week 0 versus 5 mg/l (5-54) at week 4 in luminal Crohn disease; $P < 0.0001$; 19.4 mg/l (5-134) at week 0 versus 5 mg/l (5-79.6) at week 10 in fistulizing Crohn disease; $P < 0.0001$) which was not present in non-responders (5 mg/l (5-110) at week 0 versus 7 mg/l (5-60.9) at week 4 in luminal Crohn disease: NS; 8.1 mg/l (5-39.5) at week 0 versus 7 mg/l (5-64) at week 10 in fistulizing Crohn disease; NS).

CRP level before infliximab treatment

When comparing CRP levels before treatment in the whole group of patients, we found significantly higher levels in responders than in non-responders (Table II). When looking separately at luminal and fistulizing disease. CRP levels were still higher in responders than in non-responders, but without reaching the level of significance. When studying subgroups of patients according to the location of the disease or to the use of immunosuppressives, a higher CRP level was found in responders than in non-responders in all subgroups, but the difference was significant only in colonic disease (22.0 mg/l (5-160) versus 8.5 mg/l (5-144); $P = 0.03$). When grouping patients according to their baseline CRP value in two subgroups: one with normal CRP value (<5mg/l) and the second with elevated CRP value (>5 mg/l), there were 12/26 (46%) responders in the first one and 97/127 (76%) in the second one ($P = 0.004$; OR = 0.26 (95% CI: 0.11-0.63). Logistic procedure and ROC curve disclosed no better discriminant level of CRP. This discrimination on the basis of a CRP level at 5 mg/l was more prominent in luminal disease (6/15 (40%) versus 54/70 (77%); $P = 0.009$; OR = 0.20 (95% CI: 0.06-0.64) than in fistulizing disease (6/11 (54%) versus 43/57 (75%); $P = 0.26$).

Table III: *TNF-α levels (pg/ml) expressed as median and range before infliximab treatment according to response to treatment*

	Responders	**Non-responders**
Whole group of patients (109 responders/44 non-responders)	14 (10-314)	14 (10-90.9)
Luminal Crohn's disease (60 responders/25 non-responders)	15.2 (10-314)	10 (10-32.6)*
Fistulizing Crohn's disease (49 responders/19 non-responders)	13 (10-242)	21.6 (10-90.9)

*P=0.06.

Table IV: *Allelic and genotype frequencies (%) for -308 TNF gene polymorphism according to response to infliximab treatment*

	TNF1	**TNF2**	**TNF1/TNF1**	**TNF1/TNF2**	**TNF2/TNF2**
Responders (*n* = 158)	85.8	14.2	73.4	24.7	1.9
Non-responders (*n* = 56)	78.6	21.4	62.5	32.1	5.4

Serum TNF-α level before infliximab treatment (Table III)

When comparing TNF-α levels between responders and non-responders in the whole population, we found no significant difference. However, when looking separately at luminal disease, there was a trend toward a higher TNF-α ($P = 0.06$) serum level before treatment in responders. There was no significant difference in fistulizing Crohn disease studied separately. When studying subgroups of patients according to the location of the disease or to the use or not of immunosuppressives, there was no significant difference between responders and non-

responders. When grouping patients according to their baseline TNF-α level (< 10 pg/ml or > 10 pg/ml) there was no significant difference in response rate either in the whole group of patients or in luminal or fistulizing disease separately. Logistic procedure and ROC curve found no significant discriminant level for TNF-α.

-308 TNF genotyping

Genotyping for -308 TNF polymorphism was successful in 220/226 patients. Allelic frequencies for the -308 TNF genotyping were 83.9% for TNF1 and 16.1% for TNF2. This was not significantly different from allelic frequencies in controls (85.2% and 14.8% for TNF1 and TNF2, respectively).

In 214 patients, we had both successful genotyping and evaluable clinical response. Allelic and genotype frequencies according to the response to infliximab are given in Table IV. There was no significant difference between responders (complete and partial) and non-responders. There was no significant difference either when considering complete responders only, compared to non-responders (TNF2 allelic frequencies: 16.5% and 21.4% in complete responders and non-responders, respectively; NS).

When looking separately at fistulizing Crohn disease, there was no significant difference between responders and non-responders (TNF2 frequency was 18.6% and 20.5% in responders and non-responders, respectively; NS). Allelic and genotype frequencies in luminal Crohn disease are given in Table V. There was a slight significant increase of TNF2 in non-responders ($P = 0.04$), but there was no significant difference when comparing genotypes. When grouping the patients according to location of the disease or use of immunosuppressives, there was no significant difference between responders and non-responders.

Finally, the serum TNF-α levels before treatment were not significantly different between TNF1 homozygotes and TNF2 carriers (10 pg/ml (10-314) versus 15.6 pg/ml (10-242); NS).

Table V: *Allelic and genotype frequencies (%) for -308 TNF gene polymorphism according to response to infliximab treatment in the subgroup of luminal Crohn disease*

	TNF1	TNF2	TNF1/TNF1	TNF1/TNF2	TNF2/TNF2
Responders (*n* = 99)	88.4	11.6	77.8	21.2	1
Non-responders (*n* = 34)	77.9	22.1*	64.7	26.5	8.8

*$P = 0.04$.

Discussion

In this study, both biological and genetic characteristics were examined as prognostic factors for response to infliximab. There was a positive significant association between response to infliximab and CRP level before treatment. There was also a trend toward a higher TNF-α level in responders essentially in luminal Crohn disease, but no clinically relevant association with the -308 TNF genotype.

The patients included in the present study were treated with infliximab in the setting of an expanded access programme to that treatment. The criteria of treatment in this programme were similar to those in previously published controlled trials (1,2). The presence of a clinical response was also determined according to previously published criteria (1,2). Not surprisingly, the response rate was concordant with what has been observed in treated patients in those controlled studies. Furthermore, as in the trial with luminal Crohn disease (1), we observed a significant biological response associated with the clinical response, as assessed by a decrease in CRP levels. In our patients, the significant biological response was also present in responding fistulizing Crohn disease. This associated biological response strengthens the value of our clinical evaluation. When defining the biological or genetic criteria that are associated with response to treatment, this is of paramount importance. What is striking with infliximab treatment is that, despite clinical improvement in more than two-thirds of patients, sometimes very impressive and dramatic, still one-fourth to one-third of patients do not respond at all to the treatment. Therefore, it seemed logical to isolate these non-responders as a relevant homogeneous subgroup of patients and to compare them to either partial or complete responders.

The most relevant result of our work is the higher baseline CRP level in patients who respond to infliximab. It was possible, mainly in luminal disease, to separate patients according to baseline CRP level into two subgroups

with significantly different proportions of response to infliximab. At first sight, it may seem strange that some refractory Crohn disease patients may have low and even normal CRP levels. However, these patients were often already treated with multiple anti-inflammatory and immunomodulatory drugs, probably decreasing the systemic inflammatory reaction without completely controlling the activity of the disease. Indeed, it has been shown consistently that there is no perfect correlation between clinical activity indexes and biological parameters of inflammation in Crohn disease (34). Furthermore, in our patients who were selected for infliximab treatment on the basis of clinical activity assessed either by the CDAI or by the existence of refractory draining fistulae, there was no association between CDAI before treatment and response to treatment (data not shown). We cannot therefore conclude that non-responders had actually inactive disease but rather that they were patients with clinically active disease despite a low systemic inflammatory reaction. The mechanisms of these persistently active Crohn diseases without classical systemic inflammation remain unclear, but may involve other patterns of inflammatory and immune activation (35). Interestingly, in luminal disease, the higher CRP level in responders was also associated with a higher TNF-α level. Serum TNF-α level does not have a good sensitivity for gut inflammation. Although it is generally higher in active than in inactive disease, its correlation with clinical disease activity is not strong (36) despite increased local production and even clinical significance of the increased local production (8-10). This situation may be due to the short half-life of locally produced TNF-α (37). It is therefore relevant to find higher serum TNF-α levels in patients responding to infliximab because it may represent the tip of the iceberg of a stronger TNF-driven mucosal inflammation. The fact that this trend was not found in fistulizing Crohn disease may be due to a reduced extent of intestinal lesions in a large proportion of the patients who had mainly perianal disease in comparison with patients who had more extensive luminal disease. According to this, many patients with fistulizing Crohn disease had a normal CDAI before treatment, indicating a rather quiescent luminal disease. In these patients, the amount of TNF-α reaching blood circulation was probably insufficient to be detected.

TNF-α production has been shown to depend on genetic background. In particular the -308 polymorphism in the promoter of the gene has been shown to influence TNF-α transcription (22). The carriage of the rarer allele of this polymorphism (TNF2) has been associated with a higher capacity of TNF-α production in several in vitro (23) and ex vivo (24) models, although no influence was found by some authors (38,39). In a previous study we found an increased frequency of TNF2 in severe, particularly steroid-dependent Crohn disease, although this subgroup of patients was only a small sample (30). However, as far as the response to infliximab is concerned, in the present study we did not find any association between TNF2 carriage and a positive response to infliximab which could have been suspected in view of the higher CRP and TNF serum levels in responders. We even found a lower frequency of allele TNF2 in responders in the subgroup of refractory active Crohn disease but without significant difference in genotype frequencies. Furthermore, we found no association between -308 TNF genotype and serum TNF levels in our population. These results may be interpreted either as an absence of or at best a weak (and insufficient to be detected) influence of this polymorphism on TNF production and circulating level. According to our results, -308 TNF gene polymorphism plays no relevant role in determining the response to infliximab. Other TNF gene polymorphisms may still be involved. A recent article on a rather small cohort of patients suggested an association between polymorphisms in the lymphotoxin A gene (or the adjacent TNF region) and response to infliximab (17). However, another recent study, also in a fairly small population of infliximab-treated patients has not found any association between several single base pair polymorphisms as well as microsatellites in TNF gene and the response to infliximab (40). These results should be confirmed on a larger population of patients. Beside TNF other genes should also be studied. However, the choice of relevant candidate genes would necessitate a better understanding of the mechanism of action of infliximab, which is still only partly understood. Besides blockade of soluble TNF-α (41,42), infliximab also binds membrane TNF (43). By doing this, it may induce death of the cells expressing TNF by complement activation (43). antibody-dependent cell-mediated cytotoxicity (43) or even induction of apoptosis (6). A recent article shedding some new light on the definition of response to infliximab shows that what we have been calling non-responders at 4 weeks may actually be short duration responders (44). In that study, almost 90% of patients were clinical and biological responders at 1 week, but one-third of them had a quick reactivation of TNF-α secretion and increase of NFκB in the mucosa associated with clinical activity.

In conclusion, a positive response to infliximab was associated with a higher systemic inflammatory reaction as assessed by CRP and. to a lesser extent. TNF-α serum levels, but not with a particular genotype of -308 TNF gene polymorphism. Therefore, besides the clinical activity of the disease in patients considered as refractory Crohn disease and candidates for infliximab treatment it would be justifiable to prefer this treatment in patients with high systemic inflammatory reaction, in particular elevated CRP level.

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