

Clinical and biological consequences of immunization to infliximab in pediatric Crohn's disease

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Abstract

Tumor necrosis factor (TNF)- α plays a critical role in the initiation and progression of Crohn's disease, a chronic inflammatory disorder of the gastrointestinal tract. Infliximab, a chimeric monoclonal antibody blocking TNF- α , has proven effective as an induction and maintenance therapy for refractory Crohn's disease in adult and pediatric patients. However, infliximab therapy induces the appearance of neutralizing anti-infliximab antibodies. In the pediatric cohort, we analyzed ($n = 28$) sensitization occurred in 35.7% patients and was associated with a loss of response to maintenance infusions. In two patients presenting high titers of anti-infliximab antibodies, severe infusion reactions were observed, possibly IgE-mediated, precluding further use of the medication. Serum concentrations of TNF- α and infliximab were influenced by the presence of anti-infliximab antibodies. We propose that surveillance of circulating infliximab and/or TNF- α concentration during maintenance therapy represents an indirect but reliable method to monitor anti-infliximab immunization.

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Introduction

Crohn's disease (CD) is a chronic recurrent inflammatory disorder potentially involving the entire gastrointestinal tract. Experimental colitis models as well as studies in CD patients point to a key role of tumor necrosis factor (TNF)- α in the initiation and amplification of the mucosal inflammatory reaction [1–4]. Infliximab (Remicade[®]), a mouse/human chimeric antibody blocking TNF- α , is highly effective in otherwise treatment-resistant CD in adult cohorts [5–8]. Two prospective pediatric studies have shown a decreased CD activity index in children treated by infliximab, confirming previous results in adult CD [9,10].

A major issue with infliximab therapy for active luminal or fistulizing disease is that both in adults and children,

remission very seldom lasts more than 3 months. This has prompted the evaluation of repeated infliximab infusions either on an on-demand or a regularly scheduled basis. Three different randomized controlled adult studies, as well as pediatric retrospective studies have demonstrated the safety and efficacy of infliximab as a maintenance therapy [10–14]. However, with repeated infliximab perfusions, a loss of efficacy is observed in some patients. This loss of clinical response, as well as the occurrence of immunoallergic reactions during and/or following infusions, have been related to the formation of anti-idiotypic antibodies directed against the murine hypervariable motifs of infliximab (ATI) [12,15–17]. The incidence of anti-infliximab immunization varies from 6 to 70% [18] and was recently shown to depend on the therapeutic scheme and/or concomitant use of steroids and immunosuppressive medications [17–19].

We addressed retrospectively the incidence of anti-infliximab sensitization and its clinical and biological

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consequences in a pediatric population of 28 refractory CD patients treated with infliximab. We observed ATI in 35.7% of patients. The presence of ATI was clearly associated with a loss of clinical efficacy and severe infusion reactions occurred in two patients showing the highest titers of ATI. The type of therapeutic scheme at induction appeared to impact the occurrence of anti-infliximab immunization. Following infliximab infusions, typical patterns of infliximab and TNF- α serum concentrations were observed that were lost in case of anti-infliximab sensitization. Based on these data, we propose an immunobiological surveillance in patients treated with infliximab to avoid potentially harmful immunoallergic reactions.

Methods

Patients

Twenty-eight children with refractory luminal or fistulizing Crohn's disease, 6-month- to 15-year-old (mean $\pm$ SD = 10.5 $\pm$ 3.3 years), were treated with infliximab between October 1999 and January 2004 in two different centers and were retrospectively studied. The demographics of the patients are shown in Table 1. The diagnosis of CD was based on clinical, endoscopic, histological and radiological data. Seven children had recent-onset disease (less than 18 months duration) and 21 had more than 18 months disease duration. Indication for infliximab treatment was either steroid-dependency (19 children), as defined by the persistence of an active disease for more than 3 months despite therapy with >0.15 mg/kg/day steroid and an immunosuppressive drug, or resistance to corticosteroids (9 children), as defined by the absence of clinical remission after 2 weeks of steroids at high doses (1 mg/kg/day) with an immunosuppressive drug. All infusions were administered IV over 3 h and were systematically preceded by premedication with dexchlorpheniramin and since 2002, 100 mg hydrocortisone. Patients were treated with either one infusion or a series of three infusions at weeks 0, 2 and 6, at a dose of 5 mg/kg. Patients who relapsed were retreated with infliximab either on an on-demand basis ($n = 20$) or every 8 weeks (scheduled strategy) ($n = 3$) at a dose of 5 or 10 mg/kg. Some patients ($n = 11$) crossed over from on-demand strategy to scheduled strategy during retreatment. Immunosuppressive therapies included: methotrexate (0.4 mg/kg/week), azathioprine (2 mg/kg/day), tacrolimus (0.3 mg/kg/day), thioguanine (100 mg/m²), cyclosporin (3 to 4 mg/kg/day) or mycophenolate mofetil (600 mg/m²). One single patient did not receive any immunosuppressive drug.

Disease activity was assessed clinically using the Harvey–Bradshaw Index (HBI). Response to infliximab treatment was evaluated before and after each infusion. The immediate response to induction therapy was defined as a clinical remission (HBI < 5) 4 weeks after the first infusion or the first series of infusions. Long-term response was

Table 1

Demography	
Age at onset (years)	
Mean $\pm$ SD	10.5 $\pm$ 3.3
Range	0.5–15
Duration of disease before initiation of infliximab therapy	
<18 months	7
>18 months	21
Gender	
Male	17
Female	11
Diagnosis	
Crohn's disease (Granuloma)	22
Crohn's disease likely (No granuloma)	6
Duration of disease at the end of the follow-up period (months)	
Mean $\pm$ SD	62 $\pm$ 24
Range	15–119
Histological localization	
Oeso-gastro-duodenitis	19
Duodeno-jejunitis	11
Ileitis	16
Pancolitis	23
Perianal lesions	4
Perineal fistula	15
Adjuvant immunosuppressive therapy	
Methotrexate	18
Azathioprine	16
Tacrolimus	3
Mycophenolate mofetil	3
Cyclosporin	2
Thioguanin	2
None	1
Total number of infliximab infusions	
Mean per patient $\pm$ SD	7.2 $\pm$ 3.9
Range	3–15

evaluated at the end of the follow-up of each individual patient: patients were considered non-responders if the HBI was >5 and/or if surgery had been required and/or in case of a persistent perineal fistula.

Biological tests

The serum concentrations of TNF- α and infliximab were measured on each visit. Detection of antibodies to infliximab was retrospectively performed in any available serum sample, in general 2 months after infliximab induction therapy and then before each new infusion.

TNF- α concentrations in the serum were measured using an ELISA immunoassay, according to manufacturer's instructions (Biosource Europe, SA, Nivelles, Belgium). This ELISA detects total TNF- α , i.e., free TNF- α and TNF- α bound to soluble TNF receptors p55 and p75 [20,21]. The range of values observed in healthy controls was 0–20 pg/ml.

TNF- α biological activity was measured in a cytotoxicity assay using the L-929 cell line which is sensitive to TNF- α -mediated cellular lysis [22]. Results were expressed in pg/ml, according to a standard curve established with recombi-

nant TNF- α . The range of values found in healthy control individuals was 0–40 pg/ml.

Infliximab concentration in the serum was measured using a solid phase ELISA. In brief, infliximab was captured with plate-bound recombinant TNF- α and detected with an horseradish peroxidase-conjugated anti-human IgG1 (Caltag Laboratories, California, USA). A cut-off value of 1.8 μ g/ml was defined, based on the mean value +2 Standard deviation (SD) found in the serum of 10 patients never treated with infliximab. Concentrations below this cut-off value were reported as null.

The detection of ATIs was based on the ability of anti-infliximab anti-idiotypic antibodies to neutralize infliximab activity and consequently to inhibit its binding to TNF- α . This detection could be performed only in the absence of any detectable concentration of infliximab. Serum was incubated 30 mn at 37°C with 25 ng/ml of biotinylated infliximab. Free biotinylated infliximab (unbound by ATIs present in the serum) was then captured on plate-bound TNF- α and detected with horse radish peroxidase (HRP)-conjugated streptavidin. Results were expressed as a percentage of inhibition of infliximab binding based on the OD difference between the tested sample and a reference sample composed of pooled serum from patients never treated with infliximab ($((OD_{\text{test serum}} - OD_{\text{reference}}) / OD_{\text{reference}})$). For each serum, 1:10 and 1:100 dilutions were tested. The assay was considered positive when 100% inhibition of infliximab binding was found in the 1:10 dilution. Serum still giving complete inhibition of infliximab activity in the 1:100 dilution was further titrated and results were expressed as a titer, defined as the last dilution giving significant inhibition (at least 30%). This cut-off value was chosen on the basis of non-specific inhibition found in samples from untreated healthy and diseased controls and from non-immunized infliximab-treated patients (1:10 dilution).

In one patient, ATIs of the IgE isotype were detected with biotinylated infliximab and HRP-streptavidin after capture of total serum IgE with plate-bound anti-IgE.

Statistical analysis

Analysis of contingency tables with calculation of odd ratios (O.R.) were performed using the exact Fisher's *t* test. Comparison of quantitative variables was made using the unpaired non-parametric Mann–Whitney test. Correlations between total TNF- α and infliximab concentrations were calculated using the non-parametric Spearman correlation.

Results

Immediate and long term response to infliximab therapy

Induction therapy consisted of a single infusion of infliximab 5 mg/kg (*n* = 9) or a series of three infusions 5 mg/kg at week 0, 2 and 6 (*n* = 19). All patients but one

(96.4%) came into clinical remission after induction therapy. Remission was long lasting in one patient (3.7%) who subsequently never relapsed, while all the remaining 26 children relapsed within 2 to 17 months (96.1% relapses within 1 year).

Among these 26 children, 23 were retreated with infliximab (5 or 10 mg/kg) and received on average 6 new infusions (range: 1–13). One child was lost during follow-up and clinical evaluation of patients under infliximab maintenance therapy was thus performed on 22 patients only. Maintenance therapy was discontinued in eight children because of a severe and acute infusion reaction suggestive of anaphylaxis (*n* = 2), a relapse requiring surgery (*n* = 2), or a sustained remission 4 to 29 months after the last on-demand infusion (*n* = 4). At the end of follow-up, 14 of the 22 children were still on maintenance therapy: ten of them were in clinical remission while four patients showed signs of clinical relapse.

Overall, 64% of the 22 children still benefited from infliximab therapy at the end of the study period, whereas 36% of them either had either relapsed during maintenance therapy (*n* = 6) or had experienced a severe infusion reaction (*n* = 2).

Incidence of anti-infliximab sensitization

We tested the presence of ATIs using a quantitative assay detecting only neutralizing anti-idiotypic antibodies (see Methods). Ten children (35.7%) developed ATIs during the course of treatment (Table 2). The type of induction therapy (one versus three infusions) appeared to increase the risk of developing ATIs: among nine patients treated initially with a single infusion, seven (77.7%) developed ATIs as opposed to only three (15.7%) of nineteen patients treated with three consecutive infusions (*P* = 0.0028, O.R. = 18.6) (Fig. 1a).

Among ATI+ children treated with a single infusion at induction (*n* = 7), ATIs were first detected after the first infusion in two patients and after the second infusion in two other patients. The three remaining children of this group showed ATIs following the 6th, 7th and 12th infliximab infusions, respectively.

Table 2
Characteristics of ATI-positive patients

Patients	Infliximab inhibition in the 1 :100 serum dilution (%)	ATI titer	Number of induction infusions	Severe infusion reaction
9	53%	1 :100	3	0
8	66%	1 :100	3	0
3	96%	1 :2000	3	0
10	48%	1 :100	1	0
11	56%	1 :100	1	0
7	72%	1 :100	1	0
1	91%	1 :100	1	0
2	94%	1 :1000	1	0
4	99%	1 :4000	1	1
5	100%	1 :8000	1	1

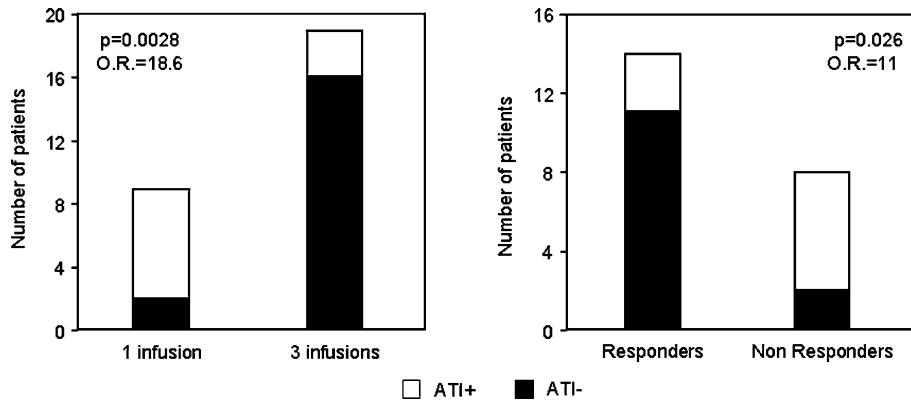


Fig. 1. Incidence of anti-infliximab immunization. In panel a, the numbers of ATI-positive and ATI-negative patients are shown in the group of children treated with a single infusion at induction ($n = 9$) and in the group treated with three infusions ($n = 19$). In panel b, the numbers of ATI-positive and -negative patients are presented in relation to clinical response at the end of the study period: responders still benefited from therapy ($n = 14$) while non responders ($n = 8$) had either relapsed during maintenance therapy or experienced a severe infusion reaction.

In children treated at induction with three infusions ($n = 3$), ATIs were detected between the 3rd and the 9th infusion.

Due to the small size of our series, no relationship could be evidenced between the incidence of anti-infliximab sensitization and the number of infliximab infusions nor the nature of the concomitantly associated immunosuppressive drug. The single child who did not receive any conventional immunosuppressant, developed ATIs detected after the second infusion and experienced a severe acute infusion reaction.

Clinical consequences of anti-infliximab sensitization

The role of ATI development in the long-term response to infliximab treatment was evaluated in 22

children who received maintenance therapy. We observed a direct relation between the loss of response to infliximab and the presence of ATIs. Among fourteen children who were still responders at the end of the study period, eleven (78.5%) had not developed ATIs, whereas ATIs could be detected in six (75%) of the eight children who did not respond to infliximab any longer ($P = 0.026$, O.R. = 11) (Fig. 1b). However, in three patients, some effect of infliximab therapy was retained despite the presence of ATIs. Two of these children (patients 7 and 10) had a titer of ATIs (1:100) insufficient to completely neutralize the activity of infliximab (as assessed by partial in vitro inhibition of infliximab in the 1:100 serum dilution: 48% and 72%, respectively) (Table 2).

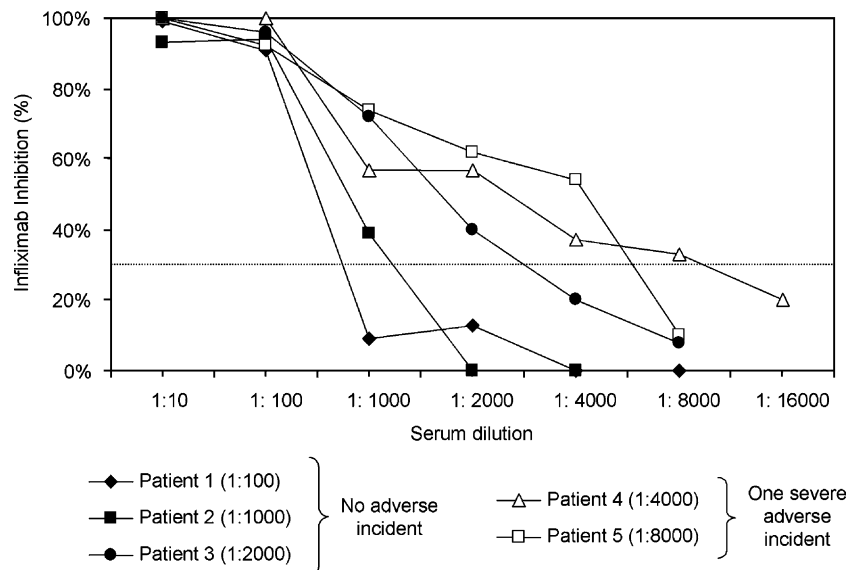


Fig. 2. ATI titers and adverse infusion reactions. Serum samples from ATI+ patients 1 to 5, characterized by a strong anti-infliximab inhibitory activity (100% inhibition in the 1:100 dilution), were titrated. The percentage of inhibition of infliximab found in each serum dilution is shown. The titer of ATIs, as defined by the last dilution giving at least 30% inhibition of infliximab, is indicated for each serum. The threshold of 30% inhibition is represented as a dotted line. Patients 1, 2 and 3 (closed symbols) remained free of any adverse incident throughout the study whereas patients 4 and 5 (open symbols) both experienced one severe infusion reaction that motivated discontinuation of therapy. ATIs of the IgE isotype were identified in patient 5.

Five patients had higher titers of ATIs, as illustrated by complete in vitro inhibition of infliximab in the 1:100 serum dilution (mean $\pm$ SEM = $96.0\% \pm 1.6\%$ inhibition) (Table 2). In two of them, significant inhibition was still detectable in the 1:4000 (patient 4) and 1:8000 (patient 5) serum dilution. These two children with high titers of ATIs were those who experienced a severe infusion reaction which motivated discontinuation of therapy (Fig. 2 and Table 2). In patient 5, we were able to detect ATIs of the IgE isotype in a sample drawn the day after the infusion reaction, suggesting the anaphylactic nature of the incident.

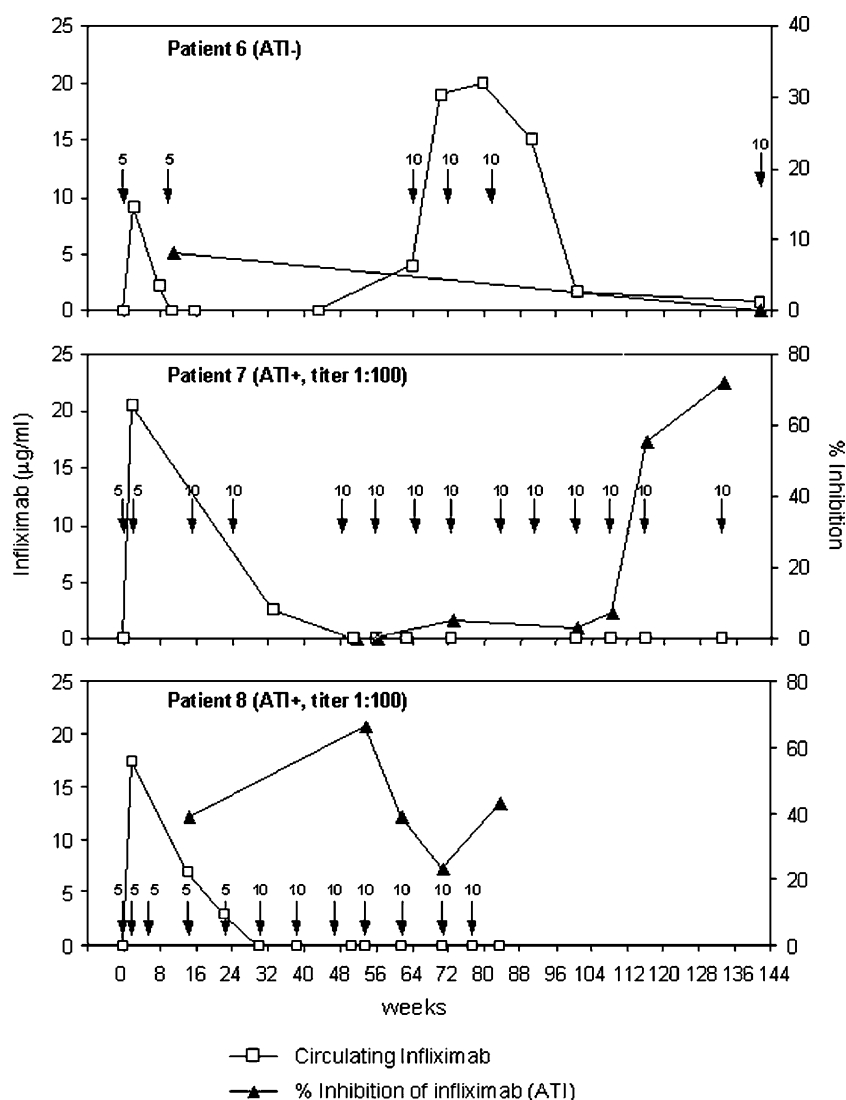
In another patient who remained ATI negative throughout the study, a mild hypertensive reaction during an infusion was noted, that resolved with treatment and did not justify discontinuation of therapy.

Overall, for a total of 205 infusions administered to 28 children, two acute immunoallergic type infusion reactions (1.0% of total number of infusions and 7.1% of treated children) were observed.

Monitoring of infliximab therapy

Circulating infliximab

Infliximab concentration was measured before each new infusion. During induction therapy, the mean residual concentration was 16.7 ± 7.3 $\mu\text{g/ml}$ at week 2 after a single 5 mg/kg infusion ($n = 20$) and 8.8 ± 7.1 $\mu\text{g/ml}$ at week 6, 4 weeks after the second 5 mg/kg infusion in patients treated with 3 injections ($n = 14$). These values are consistent with a terminal half-life of 9.5 days and a mean residence time of



13 days when infliximab is injected at a single dose of 5 mg/kg (Centocor, Remicade®, package insert and [23]). No residual concentration of infliximab could be detected 8 weeks after each infusion in patients treated sequentially at a dose of 5 mg/kg. Three patients were retreated sequentially at a higher dose of 10 mg/kg and were expected to show detectable residual concentrations of infliximab 8 weeks after the last infusion. In one of them, infliximab was indeed detected 8 weeks after each 10 mg/kg infusion (patient 6 in Fig. 3). This patient was still ATI negative at the end of follow-up. By contrast, in patients 7 and 8 (Fig. 3), no residual infliximab could be measured before each new infusion. Interestingly, in these two patients, this faster clearance of infliximab was associated with positive detection of ATIs during follow-up (Fig. 3).

Immunoreactive TNF- α

Before treatment, mean TNF- α concentration was slightly increased: 30 ± 11 pg/ml in the 23 patients tested as compared to the range of normal values found in healthy controls (0–20 pg/ml). Compared to pre-treatment levels, total TNF- α was significantly and paradoxically increased

in 96% of patients 2 weeks after the first infusion (mean: 122 ± 57 pg/ml, $P < 0.0001$) and in 67% of patients 4 weeks after the second infusion (mean: 98 ± 48 pg/ml, $P < 0.0001$) (Fig. 4b). We did not find any parallel increase of TNF- α biological activity (as measured by the L-929 bioassay) at week 2 or week 6 of induction therapy, but rather a trend towards a decrease of TNF- α biological activity (data not shown). This pattern of TNF- α was superimposable to that of circulating infliximab (Fig. 4a). We found a positive correlation between the concentration of TNF- α and of infliximab on week 2 ($P = 0.02$) and mostly at week 6 ($P = 0.0003$) (Fig. 4c and d).

During maintenance therapy, a trend towards a positive correlation between the residual concentration of infliximab and TNF- α was also observed in 27 samples drawn 2 to 8 weeks (mean $\pm$ SEM: 6.1 ± 0.6 weeks) following an infusion (data not shown, $r = 0.4475$, $p = 0.0041$). In patients retreated sequentially at a 5 mg/kg dose, some residual concentration of infliximab could be detected up to 6 weeks after an infusion, associated then with increased serum TNF- α . After 8 weeks, infliximab was undetectable and TNF- α was found within the range of concentrations

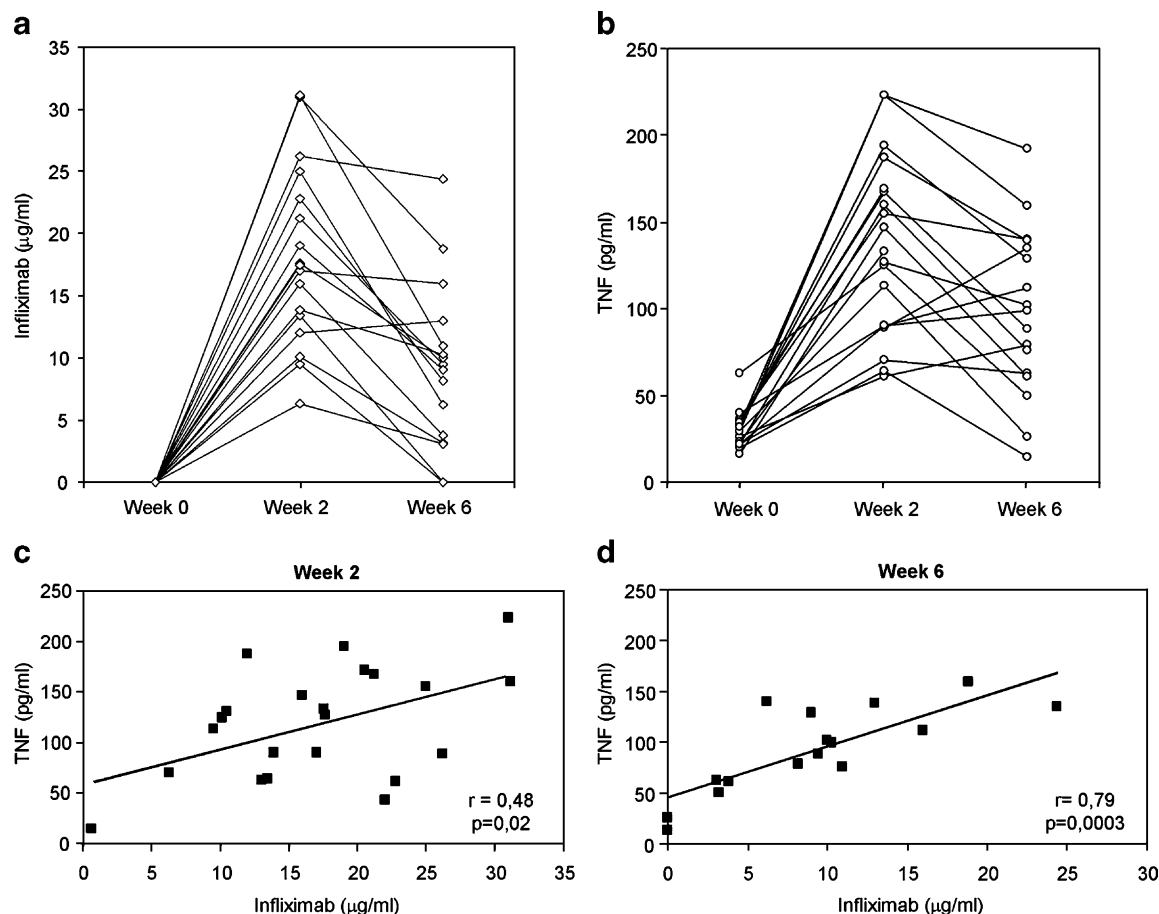


Fig. 4. Total TNF- α concentration and correlation with infliximab concentration during induction therapy. Samples from 19 patients initially treated with three 5 mg/kg induction infusions were drawn before initiation of therapy (week 0) and just before the second and the third infusion (week 2 and week 6). Dosage of circulating infliximab (a) and total TNF- α (b) was performed at each time point. Correlation between infliximab and TNF- α concentrations were calculated at week 2 after a single infusion (panel c) and at week 6 after 2 infusions (panel d). Panel c includes data from 5 patients treated initially with a single 5 mg/kg infusion.

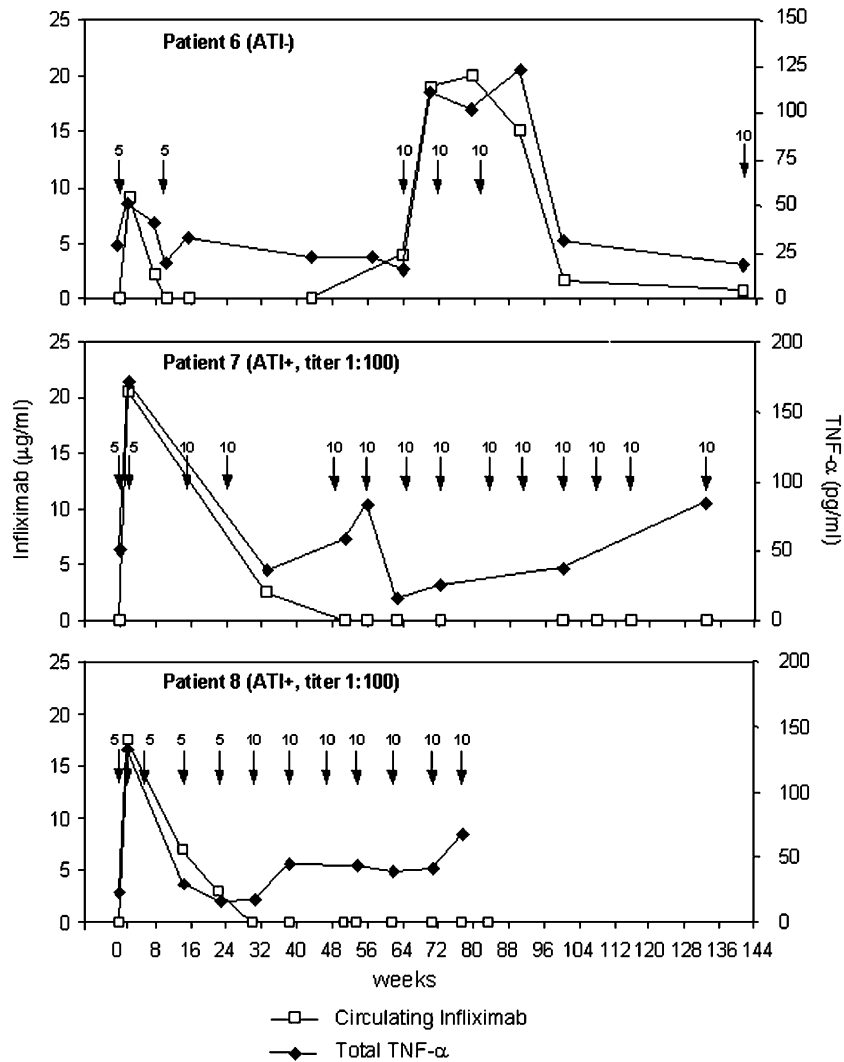


Fig. 5. Total TNF- α concentration during maintenance therapy. As in Fig. 3, dosage of circulating infliximab (left y axis) and total TNF- α (right y axis) was performed before each new infusion in patients 6 to 8. For ATI+ patients 7 and 8, the highest titer of ATI found is indicated. The time of each infusion is represented by an arrow. The numbers above the arrows indicate the dose per infusion. The scale of the x axis is set to illustrate the 8 weeks interval between scheduled maintenance infusions.

observed at baseline. In patient 6, retreated sequentially at a 10 mg/kg dose, some residual concentration of infliximab was consistently detected 8 weeks after each infusion and total TNF- α was increased (Fig. 5). This patient remained free of ATIs throughout the follow-up period. In patients 7 and 8, also retreated sequentially at a dose of 10 mg/kg, who developed ATIs and showed a faster clearance of infliximab (see above), no clear increase of TNF- α could be evidenced 8 weeks after an infusion (Fig. 5).

Discussion

One aim of this study was to evaluate the frequency of sensitization to infliximab and its consequences on the long-term response to the monoclonal antibody. We confirmed previously published data [10,24–26] showing high rates of clinical remission after infliximab induction therapy

(96.4%), high rates of relapse (96.2%) after induction therapy despite maintenance of immunosuppressants, loss of response to infliximab retreatment in a significant proportion of patients (36%) and low frequency of severe infusion reactions (1.0% of infusions in 7.1% of patients).

In our series of 28 children, the incidence of ATIs was 35.7%, similar to that recently reported in another pediatric study (35.3%) [16]. Such frequency is also within the range of that reported in adult populations (3 to 61%) [18]. Importantly, we found that a single induction dose of infliximab 5 mg/kg increases the risk of ATI formation in children, as also concluded from prospective studies performed in adults [18]. However, due to the small size of our series and the large number of patients crossing-over, we could not evaluate a potential relationship between the scheme of retreatment and the development of ATI [13,18].

The presence of ATIs was associated with a loss of response to maintenance infusions of infliximab. The

concentration of ATIs appeared to be critical as some patients with moderate titers of ATIs still benefited from infliximab therapy, despite a reduced duration of clinical remission between infusions. In contrast, high titers of ATIs in two patients were associated with severe infusion reactions, possibly IgE-mediated, precluding further use of the medication. The incidence of such infusion incidents observed by us and others [18] is rather low. Nevertheless, the frequency of sensitization to infliximab (35.7% in our series) suggests that regular detection and quantification of ATIs might be of interest to detect patients at risk for loss of clinical response and harmful immunoallergic reactions.

The detection of ATIs is hampered by the presence of circulating infliximab, being thus problematic in patients retreated on a scheduled mode (specially at a 10 mg/kg dose). In such patients, the presence of ATIs may also interfere with the measurement of infliximab, which is based on the detection of the biologically active binding sites of infliximab, not of the chimeric antibody itself (see Methods). Thus, a faster clearance of infliximab after an infusion might reflect either individual variations of the monoclonal antibody kinetics or the presence of ATIs neutralizing its biological activity.

Following an infusion of infliximab (2 to 4 weeks after a 5 mg/kg induction infusion and up to 8 weeks after a 10 mg/kg maintenance infusion), the presence of a residual concentration of infliximab was associated with an increase of immunoreactive TNF- α , but not biologically active TNF- α in the serum. Similar observations have been made in adult patients with rheumatoid arthritis [27]. A lengthening of TNF- α serum half-life when bound to infliximab could account for these observations, as shown for IL-6 during anti-IL6 therapy [28]. This seems unlikely since TNF- α /infliximab complexes are not detected by the TNF ELISA used in this study (data not shown). Another possibility is that infliximab infusions induce an increase of soluble TNF receptors p55 and p75 that are released from monocytes upon activation [29]. TNF- α /p55 or p75 complexes are indeed detected by the TNF- α ELISA used in this study [20,21]. The formation of such complexes may in fact participate to the neutralization of TNF- α biological activity during infliximab therapy.

The superimposable pattern of circulating infliximab and TNF- α 8 weeks after a 10-mg/kg infusion observed in an ATI-negative patient was lost in two ATI-positive patients: a faster clearance of infliximab was observed while no clear increase of TNF- α was found. These results suggest that infliximab might have a direct effect on immunoreactive TNF- α concentration, which would be lost in case of neutralization by ATIs.

In conclusion, given the technical limitations of ATI detection and the potential harmful consequences of anti-infliximab sensitization, we propose that surveillance of circulating infliximab and/or TNF- α concentration during maintenance infliximab therapy might represent an indirect but easy method to monitor anti-infliximab immunization.

Such monitoring should be valuable, now that the general view is that systematic maintenance therapy every 8 weeks provides, at least in adult patients, a better control of disease activity despite an increased risk of anti-infliximab sensitization [11,12]. Larger studies are in progress to test the validity of this strategy.

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