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SPECIAL ARTICLE

Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: Definitions, frequency and pharmacological aspects

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Abstract

The first ECCO pathogenesis workshop focused on anti-TNF therapy failures in inflammatory bowel diseases (IBDs). The overall objective was to better understand and explore primary non response

Abbreviations: mAbs, monoclonal antibodies; IFX, infliximab; ADA, adalimumab; CZP, Certolizumab pegol; PNR, primary non response to anti-TNF agent; LOR, loss of response to anti-TNF agent; ATI, antibodies to infliximab; ATA, antibodies to adalimumab; FcγR, Fc gamma receptor; ELISA, enzyme-linked immunosorbent assays; RIA, radio-immunoassays; ANA, anti-nuclear antibodies.

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and loss of response to anti-TNF agents in IBD. The outcome of this workshop is presented into two parts. This first section addresses definitions, frequency and pharmacological aspects of anti-TNF therapy failure, including pharmacokinetics of anti-TNF monoclonal antibodies and immune and non-immune mediated clearance of anti-TNF mAbs. The second section concerns the biological roles of TNF and TNF antagonists, including mechanisms of action of anti-TNF agents, and discuss hypothesis regarding their failures and phenomenon of paradoxical inflammation, including the potential role of TNF independent inflammatory pathways.

Contents

1. Introduction

The introduction of drugs directed against tumour-necrosis factor (anti-TNF) has greatly advanced the therapeutic armamentarium for the treatment of inflammatory bowel diseases (IBDs). Infliximab (IFX), followed by Adalimumab (ADA) and Certolizumab pegol (CZP) have shown significant efficacy in severe Crohn's disease (CD) refractory to conventional treatments, including immunosuppressive drugs.[1–3] Efficacy for fistulizing CD has also been shown in a placebo controlled trial with IFX and in a post-hoc analysis of a pivotal trial with ADA.[2,4] This clinical efficacy has been associated with mucosal healing and improvement in quality of life. The efficacy of anti-TNF agents has also been shown to exert a major impact on the outcome of important disease parameters (i.e. a reduction in hospitalizations and surgeries).[5,6] However, some patients do not respond to anti-TNF agents and a significant proportion of responders may lose response over time.

The scientific committee of ECCO has launched the first pathogenesis workshop which focused on this significant clinical problem. The overall objective was to better understand and explore primary non response (PNR) and loss of response (LOR) to anti-TNF agents in IBD.

The outcome of this workshop is presented into two parts. The first manuscript addresses definitions, frequency and pharmacological aspects of anti-TNF therapy failure, including pharmacokinetics of anti-TNF monoclonal antibodies (mAbs) and immune and non-immune mediated clearance of anti-TNF mAbs. The second manuscript focuses on the biological roles of TNF and TNF antagonists, including

mechanisms of action of anti-TNF agents, TNF independent inflammatory pathways, and paradoxical inflammation.

2. Definition and frequency of failures with anti-TNF monoclonal antibodies (Tables 1 and 2)

2.1. Primary non response in luminal Crohn's disease

In placebo controlled trials, the rate of no remission at week 4 was 80% with CZP, 67% with IFX and 64% with ADA.[7–9] These numbers were influenced by the induction regimen, mainly for ADA. The rate of PNR at week 4 was 71% for CZP, 40% for IFX and 41% for ADA.[7–9] The influence of induction regimen for ADA was not statistically significant.

In pivotal placebo controlled maintenance trials with open label induction, the maximal response rate was observed at week 12 for CZP and ADA and at week 10 for IFX. The rate of no remission at these time points was 73% with CZP,[3] 58% with IFX[1] and 50% with ADA (Abbott data on file).[2] The rate of no response was 64% and 54% with CZP when defined by a 100 and a 70 points decrease in Crohn's disease activity index (CDAI), respectively,[3] 29.2% with IFX (defined by a 70 points decrease in CDAI)[1] and 31% and 21% with ADA when defined by 100 and 70 points decrease, respectively (Abbott data on file).[2] In these trials, the response and remission rates were influenced by disease duration. For example, no response was observed in only 10% of patients having disease duration of less than 1 year as compared to 43% of patients having disease duration greater than 5 years, at week 26 with CZP.[3]

Mucosal healing has been evaluated with IFX therapy: absence of mucosal healing was found in 71.1% at week 10 and 55.6% at week 54.[10]

In strategy trials, remission without steroids reached a very high rate around 75% at week 12 with IFX. In these trials, IFX was combined for a few weeks with steroids, with or without immunosuppressive treatment.[6,11] Co-treatment with immunosuppressors was shown to decrease non response but only in immunosuppressor-naïve patients 12. There was no clear effect of immunosuppressor co-treatment in cases of immunosuppressor failures.[7–9]

In single center uncontrolled series, absence of response to induction were constantly lower than in controlled trials and ranged from 40% to 10% only.[13–16] In these series, lower non response rates were associated with immunosuppressives co-treatment, younger age, colonic disease, absence of stricture, non smoking and elevated CRP.

2.2. Primary non response in fistulising Crohn's disease

PNR to induction with IFX was 31% at 14 weeks.[17] Absence or incomplete closure at the same time point occurred in 52% of patients.[17] For ADA, data exist only for the 6 months time point, with absent or incomplete closure in 70% of patients.[2] Closure based only on clinical evaluation, does not equate definitive healing as illustrated by magnetic resonance imaging (MRI) assessment. After induction therapy with IFX the vast majority of clinical responders (8/11) had persistent inflammatory tracks on MRI.[18] Single center reports and uncontrolled series suggest that the combination of anti-TNF treatment with an appropriate drainage of perianal lesions and antibiotics may decrease non response rates.[19,20]

2.3. Primary non response in chronic active ulcerative colitis

Only IFX has currently been adequately evaluated in ulcerative colitis (UC). Absence of response to induction at week 8 was around 35% and absence of remission around 65%.[21] Absence of mucosal healing after induction was found in 40% of patients.[21]

2.4. Secondary non response in luminal Crohn's disease

Secondary non response or LOR to anti-TNF agents is defined in those patients who initially respond to anti-TNF therapy and subsequently lost clinical response. Most studies define clinical response as a reduction in CDAI of ≥ 70 from baseline and clinical remission as CDAI < 150. Secondary non responders are therefore those patients not achieving these clinical goals. For IFX, this is defined if occurring after the fourth dose (0, 2, 6 and 14 weeks). For ADA, this is defined if occurring after the induction phase which includes three injections in decreasing doses of 160 mg, 80 mg and 40 mg over a period of 4 weeks followed by 40 mg every other week for a total of 6–12 week period (to achieve maximal response). For CZP, loss of efficacy is present after the induction phase which includes three 400 mg doses at 0, 2, and 4 weeks.

Two placebo controlled trials evaluated IFX for the maintenance of remission in CD. Clinical response was defined as CDAI reduction ≥ 70 from baseline and clinical remission as a CDAI < 150. Rutgeerts et al. evaluated patients who initially responded to IFX at week 44.[22] Failure to maintain response was observed in 38% of them. The proportion of patients not in clinical remission by the end of follow up with IFX was 47%. Hanauer et al. evaluated 335 IFX responders.[1] The median time to LOR was > 54 weeks for IFX 5 mg/kg and 10 mg/kg. LOR at week 54 was observed in 61% and 42% of patients on IFX 5 mg/kg and 10 mg/kg, respectively. The proportion of patients not in clinical remission at weeks 30 and 54 were 61% and 71% respectively for IFX 5 mg/kg and 55% and 61.6% respectively for IFX 10 mg/kg. Two trials evaluated the secondary non response to Infliximab by assessing the need to intensify the dose and/or frequency of IFX treatment.[13,23] LOR was observed in 50%–54% of patients in these studies. A recent large cohort of 614 patients receiving IFX was followed up for a median of 55 months.[13] The authors reported non response rate of 21.6% by the end of follow up. Finally, a recent review of the literature by Gisbert and Panes evaluated data from 16 studies.[24] The reported LOR rates ranged between 11% and 48%. A total of 2236 patients were included in these studies, providing 6284 patient years of follow up. The mean percentage of patients with LOR to IFX calculated from these studies was 37%. Since the follow up time varied between these studies, it was suggested by the authors that the risk of losing response to IFX is better expressed as incidence per patient years of follow up. Using this calculation, the LOR to IFX was 13.1% per patient year.

Two placebo controlled trials evaluated ADA for maintenance of remission in CD. Similar to the IFX trials, clinical response was defined as CDAI reduction $\geq$70 from baseline and clinical remission as a CDAI<150. Colombel et al. evaluated patients who initially responded to ADA at week 54.[2] LOR was observed in 46% of the patients. The proportion of patients not in clinical remission at weeks 26 and 54 were 60% and 64% respectively for ADA every other week and 53% and 49% respectively for ADA every week. Sandborn et al. evaluated ADA responders at week 56.[25] The proportion of patients not in clinical remission was 21% and 17% respectively for ADA every other week and ADA weekly.

Two placebo controlled trials — PRECISE 1 and 2 evaluated CZP for the maintenance of remission in CD.[3,7] Clinical response was defined as CDAI reduction $\geq$100 from baseline and clinical remission as a CDAI<150. In the PRECISE 1 trial, the rate of secondary non responders at week 26 was 38%. The rate of clinical non remission at week 26 was 52%. In the PRECISE 2, secondary non response at week 26 occurred in 38% of patients who initially responded to induction therapy. Clinical non remission occurred in 52% of patients.

2.5. Secondary non response in fistulising Crohn's disease

One placebo controlled trial evaluated IFX in the treatment of patients with fistulizing CD.[4] Response was defined as reduction in the number of draining fistulas of at least 50% from baseline and remission was defined as the absence of draining fistulas. At week 54, 64% of the patients had loss of response to IFX manifesting as actively draining fistulas.

2.6. Secondary non response in chronic active ulcerative colitis

Two placebo controlled trials, ACTs 1 and 2, evaluated IFX for the maintenance of remission in UC.[21] Clinical response was defined as a decrease in the Mayo score of at least 3 points from baseline and clinical remission as a total Mayo score of 2 or less. In the ACT 1 trial, clinical non response at weeks 30 and 54 were 49% and 55% respectively. Clinical non remission at weeks 30 and 54 were 65% and 66% respectively. Lack of mucosal healing was observed in 50% of patients at week 30 and 55% of patients at week 54. In the ACT 2 trial, clinical non response at week 30 was 53% for IFX 5 mg/kg and 40% for IFX 10 mg/kg. Clinical non remission at week 30 was 74.4% for IFX 5 mg/kg and 64.2% for IFX 10 mg/kg. Lack of mucosal healing was observed in 54% and 43% of patients on IFX 5 mg/kg and 10 mg/kg respectively.

2.7. Prevention of anti-TNF therapy failure

Published data from referral centers presenting the rates of response to anti-TNF in routine practice have shown higher response rates than in controlled trials reaching 60–90% of response. These data suggest that an appropriate selection of good candidates to anti-TNF therapy give better results, but can also stem from different response definitions in clinical trials versus everyday practice, or from a different patient population with more severe disease enrolled in clinical trials. In the SONIC study, patients with active lesions at endoscopy had higher rates of response to IFX and azathioprine.[12]

The use of immunosuppressors (azathioprine, 6-mercaptopurine and methotrexate), in conjunction with IFX has been shown to significantly reduce the proportion of patients with antibodies to IFX (ATI), possibly leading to a more favourable response and reduced need for dose escalation.[24,26,27] More recently, results from SONIC study, demonstrated higher maintenance of remission rates at 6 months in the combination arm of IFX and azathioprine. Immunosuppressors seem to protect against the induction of antibodies to ADA (ATA) and antibodies to CZP as well.[3,7] One placebo controlled trial demonstrated that intravenous hydrocortisone administered in a dose of 200 mg immediately prior to IFX infusion, significantly reduced formation of ATI; 26% versus 42% in the placebo arm.[28] It is not clear however, whether this approach impacts long term effects on LOR.

Results from several studies have demonstrated that regularly scheduled IFX infusions are associated with a decreased likelihood of ATI formation. Intermittent therapy may predispose to formation of anti-drug antibodies and increased LOR.[29–31] On the other hand, Zabana and Cabre found no difference in LOR between patients receiving scheduled IFX maintenance therapy to those reintroduced to IFX after a period of 4 months of no therapy in patients who received the original 3 infusion induction regimen (15% versus 10% respectively), suggesting that this issue needs further evaluation.[32]

3. Pharmacokinetics of anti-TNF mAbs (Tables 3 and 4)

Serum half lives vary between the anti-TNF agents, when administered in humans. Murine mAbs possess much shorter half-life (2–3 days) compared to chimeric (8–10 days) and humanized (20–23 days) mAbs. Etanercept has the shortest half-life (4 days) while ADA and Golimumab exhibit a half-life between 10 and 20 days. Elimination of therapeutic proteins varies between individuals and is most likely influenced by immunogenicity (anti-drug antibodies), concentration of target antigens, FcγR polymorphisms as well as by differential clearance.

The pharmacokinetics of these agents is determined by three basic factors: (1) the mode of administration (intravenous vs. subcutaneous), (2) drug half-life and (3) peak-to-trough serum concentration. All these factors determine the therapeutic window, introduced as a concept by Nestorov in 2005.[33] The therapeutic window concept postulates that a threshold trough serum concentration is required for therapeutic efficacy. However, supra-therapeutic serum concentration may increase the hazard of infections or malignancy. The importance of a high peak concentration as a consequence of intravenous administration for efficacy and safety of anti-TNF agents in CD and UC has not been established. Peak concentrations after IFX infusion are at least 50 times higher than trough concentrations (100–300 μg/mL vs. 1–10 μg/mL). This ratio is less prominent in subcutaneously administered agents like ADA, CZP and Etanercept. When administered at a dose of 40 mg every

other week in patients with rheumatoid arthritis and CD, the trough serum concentrations of ADA range between 4 and 8 μg/mL.

The volume of distribution of IFX and ADA is comparable, which means that these molecules spread similarly into body compartments. It is unclear if this also implies that the penetration in different tissues, such as inflamed gut mucosa, is also similar. To our knowledge, distribution data for CZP are not available.

3.1. Importance of pharmacokinetics for the efficacy of anti-TNF therapy

When recommended doses are used, one can assume that initially adequate trough serum concentration is obtained in most patients and that low initial concentration is not the reason for PNR. However, data testing this hypothesis are scarce. In the original dose ranging induction trial with IFX, a dose response association has not been reported.[8] Similarly in UC patients, IFX was not superior when given at a dose of 10 mg/kg compared to the 5 mg/kg.[21] However, in the first dose ranging trial with ADA in CD, a dose/response relation was apparent.[30] Nonetheless, in all these trials, the relevance of early trough serum concentration for individual responses was never reported.

Trough serum concentration of therapeutic antibodies is probably more relevant for secondary LOR. The development of anti-drug antibodies is intrinsically linked with the use of therapeutic proteins.[34] However, in clinical practice, only antibodies which interfere with drug efficacy (neutralizing antibodies) or instigate adverse events really matter.

Drug trough serum concentration is reliably assessed regardless of anti-drug antibodies and also reflects the degree of drug degradation. Therefore, this concentration may represent a more clinically relevant surrogate marker for LOR. IFX trough serum concentration correlate with the presence of ATI and with duration of response, but this correlation is not absolute.[29,35] Also, a decrease in drug levels may be driven by mechanisms other than the induction of anti-drug antibodies. For patients with IBD, more relevant than the underlying mechanism of decreased trough serum concentration is their chance of needing accelerated dosing due to secondary LOR. This information may be inferred from clinical trials. However, it is important to note that in the long term trials with IFX, patients increased the dose in case of LOR whereas with ADA, a shortening of dosing interval was used to enhance drug exposure. In the first maintenance trial for luminal CD with IFX, ACCENT 1, 30% of patients treated with 5 mg/kg iv stepped up to the higher dose group of 10 mg/kg after 1 year because they experienced a disease flare.[1] In the maintenance trials with ADA, CHARM and CLASSIC II, the percentage of patients that shortened their dosing interval to 40 mg weekly after 1 year was 27% and 46% respectively.[2,25] In the long term maintenance trial with IFX for fistulizing CD, ACCENT 2, 25% of patients increased the dose to 10 mg/kg because their fistulas started draining again.[36]

3.2. Treatment optimization in LOR

If despite optimizing the treatment strategy, the efficacy of an anti-TNF agent fades in a patient with initial response, treatment flexibility is needed to counteract LOR. The two main strategies available are: (1) increasing drug exposure by shortening the dosing interval or increasing the dose and (2) switching to another drug. To some extent, the therapeutic intervention needs to be tailored to each individual patient.

To justify the first option of dose escalation, we need evidence that low trough serum concentration is associated with LOR and that increasing drug exposure restores efficacy. In the ACCENT 1 trial, increasing the dose from 5 to 10 mg/kg and from 10 to 15 mg/kg restored response in 62% and in 69% of patients respectively.[1] Conversely, in a single center patient cohort in Leuven of 547 patients with CD, 66% (75/108) regained clinical response by the end of follow up after having shortened their dose interval (Schnitzler 2009). Data in patients with IBD and with rheumatoid arthritis suggest that IFX trough serum concentration below 1 μg/mL correlate with LOR.[35,37] In a retrospective cohort of CD patients at the University of Toronto, ATI formation correlated with low trough concentration, CRP and the absence of long term remission.[35]

In a prospective immunosuppressive withdrawal trial, patients with CD and with low IFX trough serum concentration (below median) had higher CRP values and CDAI scores than those with trough concentration above median.[38] Hence, even if there is no absolute correlation between trough serum concentration, ATI and the clinical response, increasing drug exposure with an intention to restore trough concentration to therapeutic values is a valuable strategy. Data regarding the influence of trough serum concentration on therapeutic efficacy has not been released from the controlled trials that led to the market authorization of ADA and CZP.[2,3,7,9] However, in a retrospective cohort of CD patients treated with ADA at the University hospital of Leuven, trough serum concentration was linked to therapy discontinuation. More interestingly, in patients who regained clinical response after dose adjustment, the increment of ADA trough serum concentration was higher than in those who failed to restore response.[39] Similar data were already reported with the use of ADA in patients with rheumatoid arthritis.[40]

The strategies of dose escalation have been very different in clinical trials conducted with the different anti-TNF agents IFX, ADA and CZP. Therefore, it is impossible to choose between shortening dosing interval and increasing the dose based on clinical trial experience. For ADA the European label suggests dose intensification only by shortening the interval between injections, but for IFX both options are being employed in clinical practice. A post-hoc analysis of the pharmacokinetic data collected in the ATTRACT maintenance trial with IFX in patients with rheumatoid arthritis, suggests that shortening the interval will lead to higher trough serum concentration than increasing the dose.[37]

In case of LOR despite optimization, other therapeutic options, including switching to another anti-TNF is an option. In the GAIN trial, specifically designed to include patients with LOR or intolerant to IFX, remission rates 4 weeks after an induction dose of 160/80 mg ADA were lower when compared to those found earlier in the dose finding clinical trial, CLASSIC 1, which included patients naïve to anti-TNF therapy.[9,41] This observation needs to be confirmed, but recent clinical trial data with both ADA and CZP indicate that prior exposure to IFX attenuates the response to a second

anti-TNF agent. The reason for discontinuing a first or a second anti-TNF mAb (PNR, LOR and/or intolerance) does not seem to influence the rate of response to a second or a third anti-TNF.[41–43]

4. Immunogenicity of anti-TNF mAbs (Table 5)

4.1. Anti-TNF agents have different degree of humanization

All anti-TNF agents are compounds produced by biotechnology that mimic molecules found in the body, such as proteins and oligonucleotides. Due to their molecular nature all these agents need to be parenterally administered. Several strategies have been followed in drug development to improve the efficacy and tolerability of biological agents. Progress in protein engineering has resulted in the replacement of immunogenic non-human peptide sequences from human ones, a technique called humanization.[34,44] Third generation, humanized antibodies (±95% human) (exhibiting only murine Complementarity-Determining Regions, CDRs, and a few mouse amino-acids in the VH and VL frameworks) and fourth generation, fully (100%) human mAbs, are usually considered less immunogenic as compared to chimeric (75% human, the VH and VL being of murine origin) mAbs such as IFX. Anti-TNF agents currently available differ in their degree of humanization. Furthermore, their degree of immunogenicity is still unclear, due in part to the fact that the methods of detection of antibodies against anti-TNF antibodies vary among different studies.

4.2. Methods measuring ATI

Initial measurements for detecting ATI were mostly performed using solid-phase enzyme-linked immunosorbent assays (ELISA). This technique has a major disadvantage because standard detection antibodies (e.g. labelled anti-human Fc) used for the detection of anti-drug antibodies, may also bind with the IFX human Fc moiety in these particular assays. To overcome this problem, a sandwich ELISA has been employed by several groups as well as by a commercial manufacturer (Prometheus Laboratories, San-Diego, CA, USA). In this technique, plated IFX serves as the antigen, and is used again, in a biotinylated form, to detect anti-drug antibodies bound to the plated IFX.[29,45] However, this alternative ELISA method has also several limitations. It can detect only ATI that remain capable of binding to soluble biotinylated IFX while being already bound to IFX-coated plates (i.e. remaining divalent or polyvalent antibodies). In addition, epitope masking in the plated IFX may lead to false-negative results and the presence of soluble IFX in the serum may compete with the plate-bound one for the binding of ATI. In addition, spontaneously occurring anti-IgG antibodies (rheumatoid factor) as well as other low-affinity antibodies may bind non-specifically to the plate-coated IFX , yielding a false positive assay result.[46]

The limitations of the sandwich ELISA have lead to the development of alternative methods. A functional assay assessing the capacity of patient sera to neutralize binding of IFX to solid-phase TNF has been developed, but it does not allow the detection of non-neutralizing anti-TNF antibodies.[47]

Fluid phase assays comprising radio-immunoassays (RIA) have been also developed for ATI measurement. In general, fluid phase RIA recognize ligands with highly conserved conformations and are therefore less influenced by artefacts due to formation of new epitopes or loss of epitopes occurring after coating/coupling of proteins to solid-phase matrices. This technique allows a useful correlation with clinical response to IFX.[46,48–50] A further advantage of fluid phase RIA is that functional monovalent ATI are detected, such as IgG4, which are not measured by sandwich ELISA, but nevertheless constitute a significant amount of ATI in patients with rheumatoid arthritis.[46] On the other hand, fluid phase RIA technology does not circumvent the interference stemming from the presence of IFX in serum and is still limited for detecting only lambda-chain containing ATI, which have been shown to comprise 50% of the total IFX-ATI immune complexes in serum.[46] Other investigators used agarose-immobilized protein A to capture serum immunoglobulins and then measured radioactivity after addition of I^{125} labelled pepsin-treated IFX.[51] However, this method cannot overcome the presence of IFX in serum, and may also underestimate ATI other than IgG1 and IgG2, as the latter are preferentially captured by protein A.

4.3. Methods measuring ATA

One method to measure ATA consists of adding radio-labelled pepsin-digested ADA (i.e. the F(ab)'2 fragment of Adalimumab) to Protein A-captured serum immunoglobulins, with subsequent measurement of Sepharose-bound radioactivity.[40] Others have measured ATAs using sandwich ELISA technique, whereby unlabelled ADA serves as the bound antigen, and labelled ADA is employed in the detection phase.[52] A fluid phase RIA has also been developed.[53] The readouts of this technique were shown to correlate with clinical response to ADA, or lack hereof, in patients with rheumatoid arthritis and, most likely, with IBD. Since all these methods are similar to those used for ATI as described above, they also share similar technical limitations.

4.4. Immunogenicity and IFX

4.4.1. Allergic reactions

Acute infusion reactions need to be differentiated from delayed reactions. Acute reactions are defined as reactions occurring during or within 2 h of an infusion. They can be severe or not. Severe reactions are usually defined as reactions necessitating discontinuation of the infusion due to significant dyspnoea or drop in blood pressure. Mild to moderate acute reactions may include fever, slight decrease in blood pressure, erythema, itching, rigor or shivering.

Delayed reactions occur 2 days to 2 weeks after reinfusion of IFX. The symptoms can be quite severe and usually last 3–5 days. Delayed reactions are usually attributed to serum sickness like reactions. Possible symptoms include a cluster of features (generalized stiffness, myalgias, arthralgias, fever, and/or rash).

The main hypothesis behind these allergic reactions, acute or delayed and severe or not, is that they are related to

some form of immunogenicity against IFX. However this has not been adequately studied and the only biological marker available to assess immunization against the drug are the so-called ATI.

4.4.2. Clinical relevance of immunogenicity and IFX

In all registration studies with IFX, ATI have been detected in 4 to 38% of patients (36,55). In the early post-marketing clinical experience, up to 25% of patients developed moderate or severe infusion reactions when IFX was used on demand with and without concomitant immunosuppressive therapy.

Since then, hallmark studies have shown a relationship between ATI and infusion reactions. In a cohort of 125 consecutive patients with CD who were treated with episodic IFX infusions in the University hospital in Leuven, a correlation between IFX and ATI concentrations with clinical efficacy, side effects (including infusion reactions), and the use of concomitant medications before and 4, 8, and 12 weeks after each infusion was investigated.[29] ATI were detected in 61% of patients and in almost all of them these were developed after the first or second infusion. The cumulative incidence of infusion reactions was 27% and the vast majority of these reactions occurred during the second or third infusion. There was a strong correlation between the concentration of ATI and the occurrence of infusion reactions. The median concentration of ATI was 20.1 μg/mL (95% CI 3.0–22.6) at the time of a first infusion reaction, as compared with 3.2 μg/mL (95% CI 1.6–4.9) among patients without an infusion reaction ($p<0.001$). ATI concentration ≥ 8 μg/mL predicted a higher risk of infusion reactions (RR 2.40; 95% CI 1.65–3.66; $p<0.001$).

The median Infliximab concentration 4 weeks after an infusion was significantly lower among patients with an infusion reaction than among patients who never had a reaction (1.2 μg/mL vs. 14.1 μg/mL, $p<0.001$). A significant relation was also found between the serum IFX concentration measured 4 weeks after an infusion and the concentration of ATIs before that infusion ($r=0.34$, $p<0.001$). Once an infusion reaction occurred, the median duration of response to an infusion was shorter: 38.5 days (95% CI 34–51 days), as compared with 65 days (95% CI 56–71 days; $p<0.001$). Logistic regression analysis showed that the presence of ATI was independently associated with a shorter duration of response ($p<0.001$). Patients who were taking immunosuppressive agents had a lower incidence of ATI compared to those who were not taking such agents (43% vs. 75%) ($p<0.01$).[29]

In another cohort of 53 patients, an incidence of ATI of 36%, including all 7 patients with severe infusion reactions, was found (28). The median ATI concentration in these patients was 19.6 μg/mL. Eleven out of 15 patients (73%) who lost response to IFX therapy were ATI positive compared to none of 21 continuous responders. In addition to concurrent use of immunosuppressive therapy, the administration of a second infusion within 8 weeks from the first was protective against ATI formation. In a subsequent study in the same cohort, 80 patients were randomised to receive 200 mg of hydrocortisone or placebo before each infusion. A lower incidence of ATI was found among steroid pre-treated subjects (26% vs. 42%). In another prospective study it was demonstrated that patients receiving immunosuppressive therapy had lower ATI formation compared to those receiving IFX monotherapy (10% and 18%, respectively; $p=0.02$).[54]

Sequential measurement of ATI concentration through the ACCENT 1 study has shown that ATI may develop at any time during scheduled or episodic retreatment.[54] However, ATI formation is more pronounced in patients treated episodically than in those treated in a scheduled manner, being around 30% after 72 weeks in the episodic strategy as compared to 10% and 7% in the maintenance strategy with 5 mg/kg and 10 mg/kg, respectively. Important information provided by ACCENT 1 is that patients positive for ATI at any time point may later become negative and that globally, the proportion of patients positive for ATI at each time point is not increasing over time, even with the episodic strategy. However maintenance therapy has proven superior to episodic treatment for various reasons. The most important advantages of maintenance therapy over episodic treatment include better response and remission rates, more thorough mucosal healing, and better quality of life and reduced number of disease-related surgeries and hospitalizations. Recently the SONIC trial comparing IFX alone versus IFX plus azathioprine versus azathioprine alone maintenance therapy has shown remarkable and durable superiority for the combination therapy of IFX with immunosuppressant over an IFX maintenance regimen alone in immunosuppressive naïve patients.[12] The combination treatment yielded higher IFX concentrations and a lower incidence of ATI compared to infliximab maintenance monotherapy.[12]

4.5. Immunogenicity and ADA

4.5.1. Allergic reactions

ADA has been rarely reported to be related with systemic or injection site allergic reactions. These reactions can be drug- or host-specific and some of them seem to be IgE-mediated. In clinical trials with ADA, approximately 1% of patients experienced allergic reactions such as allergic cutaneous eruptions, anaphylactic reaction, non-specified drug reaction and urticaria. In addition, anaphylaxis and angioneurotic edema have been reported rarely in post-marketing experience with ADA. Systemic allergic reactions clinically expressed as asthma have been also reported.[55]

4.5.2. Clinical relevance of immunogenicity and ADA

ADA appears to be less immunogenic than IFX, in accordance to its human nature.[34,56] The formation of human anti-human antibodies has been already reported long ago[52,57] however, it still remains unclear which part of ADA induces anti-human antibody response.[58]

In the CLASSIC-I trial concomitant therapy with azathioprine and 6-mercaptopurine did not produce a significant change in serum concentrations of ADA.[9] The CHARM[2] and the CLASSIC-II[25] studies have reported ATA formation in 2.8% of CD patients irrespective of concomitant immunosuppressive therapy. However, the CLASSIC II study was not powered nor designed to demonstrate the protective role of azathioprine, or methotrexate in the occurrence of ATA. In addition, attempts to modulate the development of antibodies to anti-TNF therapies through concomitant immunosuppression do not necessarily prevent the need for dose escalation and/or dose interval shortening. In the CLASSIC II trial, among the patients

who developed ATA, 3/7 (43%) and 2/7 (29%) were in remission at weeks 24 and 56 respectively.[25]

In a recent study from the Leuven cohort of ADA patients, who comprise at present the largest single center cohort examining the relationship between ADA therapy, ADA trough serum concentration and ATA formation, the great majority of patients with undetectable trough serum concentration also display ATA. These antibodies were detected in 9.2% of the patients. Concomitant immunosuppressive therapy at baseline did not decrease the development of ATA. Also, pre-existing ATI did not affect subsequent response rate to ADA therapy or ATA formation.[39] ATA were also associated with non response to ADA in another study of 30 CD patients previously exposed to IFX.[59] In this study, 57% of patients receiving ADA after IFX discontinuation were ATIs positive. ATA were detected in 5/30 (17%) patients and 4 out of these five patients did not respond to ADA therapy. The presence of ATA was associated with low trough serum ADA concentration. According to this study, patients previously treated with IFX exhibiting high levels of ATI demonstrate a subsequent lower response rate to ADA than patients with low levels of ATIs, a conclusion that is in contrast with the data from the study from Karmiris et al.[39]

4.6. Limitations in measuring ATI and ATA

Notably, not only are the techniques for measurement of anti-drug antibodies different but even the results obtained by the different methods are not reported in a uniform or standardized manner that would enable reproducibility across studies. Thus, some studies report antibody levels in arbitrary units according to serial dilutions of a reference serum, whereas others report measurements in microgram/mL. Moreover, there are hitherto no studies directly comparing the different methods outlined above, and thus it is hard to draw firm conclusions as to the most accurate and/or clinically beneficial method of detection. Such comparative studies are needed in order to ascertain the best methodology for anti-drug antibody detection in terms of reproducibility, accuracy, and correlation with loss of clinical response to anti-TNF agents. Also, the real impact of ATI or ATA in the mechanisms of the early and late allergic reactions and LOR to these drugs deserves further studies before firm conclusions can be drawn. Furthermore, the formation of these immune complexes (anti-TNF IgG1/anti-IgG immune complexes) probably accelerates the clearance of mAbs through capture by cells expressing FcγRs. So far, little is known regarding the fine mapping of antibody specificity against anti-TNF mAbs.

5. Immune and non-immune clearance of anti-TNF mAbs

Clearance of mAbs is a multi-factorial process, involving different mechanisms that are either antibody-dependent or host-dependent. The elimination of IgG is known to be concentration dependent, where half-life decreases as a function of increasing serum IgG concentrations. Catabolism is the dominant elimination mechanism of mAbs. However, the exact anatomical locations of this process have not been identified.[60,61] Specific binding sites on the Fc domain of the mAb that interact with the FcRn and the Fcγ receptors seem to play a crucial role. The impact of the Fab domain on clearance depends on the targeting antigen, namely if it is a soluble or a membrane-bound one.

Table 1 Primary non response.

Key messages
1. Almost a third of patients do not show response and 2/3 do not achieve remission.
2. However, when selecting only patients with active CD (assessed by inflammatory markers and/or lesion assessment), the absence of response is rare and ranges between 10 and 30%, while it is around 40% in UC.
3. Maximal response rate is reached after 12 weeks.
4. A broad range of "response intensity" exists; full response characterized by clinical remission and tissue healing only occurs in a minority of patients (around 30%).
5. Response rate may be influenced by disease location, duration and type, active inflammation, strictures, anti-TNF dose, smoking and co-treatment.

Questions to be addressed in the future
1. What is the best definition of non response (criteria, timing)?
2. What is the optimal induction regimen (dose, number and frequency of dosage)?
3. What is the real benefit of co-treatments (for clinical efficacy, healing)?
4. What are the response rates when treating stricturing CD? Are there predictive factors of response?
5. What are the response rates in refractory proctitis?

Table 2 Secondary non response.

Key messages
1. Loss of response varies from around 50% per year in placebo controlled trials to a slightly more than 10% per year in smaller studies and monocentric experiences in which treatment optimization (including dose escalation and dose interval changes) is allowed.
2. Factors that may prevent loss of response include steroid premedication, immunosuppressive co-treatments, and maintenance treatment as opposed to episodic treatment.
3. Treatment optimization with increased dose or shortened interval allowed recovering response in 50–90% of the patients.
4. The optimal method for dose optimization is yet to be determined.

Questions to be addressed in the future
1. What is the best definition of loss of response?
2. What is the impact of induction regimen on long term response and risk of loss of response?
3. What are the best optimization regimens (dose increase, interval shortening, re-induction or co-treatment)?
4. Can the findings for infliximab be extended to the other (humanized) anti-TNF agents?

Table 3 Pharmacokinetics.

Key messages
1. Elimination of monoclonal antibodies varies between individuals and is most likely influenced by immunogenicity (anti-drug antibodies) and by differential clearance.
2. The therapeutic window concept postulates that a threshold trough concentration is required for therapeutic efficacy.
3. The pharmacokinetics of monoclonal antibodies is determined by three basic factors: the mode of administration, drug half lives and peak-through concentrations in serum.
4. The serum level of the monoclonal antibody is significantly affected by antibody formation.
5. Loss of response to anti-TNF agents is only partly explained by antibody formation and immunogenicity; other factors including individual differences in drug clearance are likely to play a role as well.

Questions to be addressed in the future
1. What is the correlation between concentrations of the anti-TNF agent in the serum and in the inflamed tissue?
2. Are factors other than immunogenicity influencing levels of anti-TNF in the blood?
3. Can the interplay between monoclonal antibodies and antigens (i.e. antigen saturation and distribution) affect IgG catabolism?

5.1. The neonatal Fc receptor (FcRn): the salvage pathway

The neonatal Fc receptor (FcRn) is a major histocompatibility complex class-1-related receptor exerting a protective role regarding IgG catabolism. This specific intestinal transport receptor not only mediates neonatal IgG absorption, but also regulates IgG homeostasis.[62] Mice genetically lacking expression of FcRn demonstrated rapid IgG elimination with a rate increased up to 10–15 fold, while no change was observed in the elimination of other immunoglobulins.[63,64] Fab fragments that lack the Fc domain making them incapable for FcRn binding, demonstrate shorter half lives than intact mAbs, although the presence of the PEG molecule also affects half-life. IgG binds FcRn via the Fc portion, remaining in this complex steady state as long as intracellular pH is mildly acidic and being released at physiologic pH.[65] Engineered mAbs should be delivered in very large doses in order to significantly alter serum IgG concentration, due to the large quantity of endogenous IgG that is present in the body. On the other hand, they demonstrate altered (usually increased) affinity to human FcRns and thus altered (usually decreased) elimination rates especially through mutation of IgG Fc residues.[66,67] Human FcRn selectively binds human IgG and this condition could explain the rapid clearance of murine IgGs from human circulation.[68] Human IgG 1, 2 and 4 exhibit longer elimination half lives (~3 weeks) than IgG3 (1 week) due to a higher affinity to FcRn.

Table 4 In case of loss of response, drug trough levels and antibody measurements could aid in decision making.

1. In patients with undetectable drug levels, antibody measurement may be useful. Most will likely have high anti-drug antibody titers and switching the drug is probably the best option in this case.
2. In patients with low to intermediate drug readouts, and absence of high titer antibodies, an attempt to restore trough levels by dose escalation or shortening infusion/injection intervals should be considered.
3. In patients with symptoms suggestive of active disease despite high trough levels, disease reassessment including the use of CRP, fecal calprotectin, and/or imaging should be performed.
4. If these patients have active inflammation and no infection, use of a compound with another mechanism of action should be considered.

Table 5 Immune and non-immune clearance.

Key messages
1. Anti-drug antibodies can lead to loss of response by increasing drug clearance.
2. Anti-drug antibodies are probably under-detected due to technical shortcomings and imperfect test timing.
3. Monoclonal antibody humanization reduces antigenicity, but is inferior to homology. Human antibodies may be also immunogenic.
4. "Neutralizing" anti-idiotypic antibodies could lead to a complete or partial inhibition of the anti-TNF mAbs binding to TNF.
5. Scarce data exist on the role of PEG-linked molecules in clearance of biologic agents consisting of Fab fragments.
6. Applying site-directed mutations within the Fc region could influence the interplay between the monoclonal antibody and FcRn or Fcγ receptors.

Questions to be addressed in the future
1. What causes formation of antibodies to anti-TNF monoclonal antibodies in some patients but not in others?
2. How could we explain the differences between patients with high and low concentrations of anti-drug antibodies?
3. What is the relative role of anti-drug antibodies on loss of response?
4. What is the preferred technique to measure anti-drug antibodies?
5. How to prevent anti-drug antibodies formation? What is the risk/benefit ratio of concomitant treatments?
6. How should anti-drug antibodies presence direct our management?
7. Can optimization of the pharmacokinetic properties of monoclonal antibodies produce more efficient molecules regarding catabolism?

5.2. Role of Fcγ receptors in the clearance of anti-TNF mAbs

FcγRs belong to the immunoglobulin superfamily and induce phagocytosis and destruction of opsonized microbes via complement dependent or antibody-dependent cell-mediated cytotoxicity. This family includes several different isoforms, namely FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIIA (CD16a) and FcγRIIIB (CD16b), which differ in their antibody affinities due to their different molecular structure. FcγRI demonstrates the highest degree of affinity with the IgG and FcγRIIB the lowest.[60,61] On the other hand, different IgG isotypes such as IgG1, 2, 3 and 4, demonstrate unique recognition and activation profiles, when interacting with various FcγRs.[69] The above mentioned characteristics regarding interaction between different FcγRs with different IgG isotypes could also affect pharmacokinetics and clearance of the IgG mAbs from the cells of the reticulo-endothelial system. For example, homozygous FcγRIIIA-F/F158 polymorphism led to more rapid elimination of opsonized red blood cells coated with an anti-D IgG3 mAb by phagocytic cells in humans.[70]

Immune complexes containing mAbs can be eliminated through interactions with FcγRs. Different couples of immune complexes can be formed, made of TNFα and mAbs, or of mAbs and anti-mAbs (ATI or ATA). The clearance efficacy is likely related to the FcγRII and FcγRIII polymorphisms, hence leading to various clinical consequences depending on the patient.

5.3. Interaction of the mAbs with the target antigen (TNFα): role of the variable region

Interaction with the target antigen can affect the elimination rate of mAbs. This condition is dose-dependent. Low mAb concentrations that do not saturate the antigen, demonstrate shorter half-life and subsequently a higher clearance rate compared to endogenous IgG; as the mAb's dose is increased and the antigen is progressively saturated, an increase in half-life and decrease in clearance rate is observed.

Monoclonal antibodies targeting soluble antigens usually interact with the FcRn and undergo a non specific clearance by the reticulo-endothelial system. Monoclonal antibodies interacting with membrane-associated internalizing antigens demonstrate a different elimination process characterized by internalization of the antibody–antigen complex, followed by degradation of the complex. In this case, the contribution of the antigen to mAb's clearance depends on antigen concentration and distribution as well as internalization and turnover rate.[60,61]

5.4. Other factors associated with mAbs clearance

Apart from the above mentioned importance of the different moieties of the mAb molecule in their clearance, other factors have also been implicated in influencing this process. These include: (1) Glycosylation process and susceptibility to proteolysis: most of the mAbs are produced as recombinant glycoproteins in eukaryotics cells and although IgG glycans represent only 3% of the total IgG molecule mass, glycoforms may impact the plasmatic clearance of the linked mAb; (2) patient's characteristic like age, gender, body weight, body surface area and existence of a co-morbidity—i.e. diabetes; and (3) concomitant treatment: a relationship between the presence of methotrexate and a decrease in ADA clearance has been implicated in rheumatoid arthritis patients.[71] Methotrexate has been shown to be a potent inducer of the expression of the FcγRI on monocytes in these patients.[72]

Conflict of interest

The following authors have no conflicts of interest: A Klein, J Van der Woude, R Eliakim, K Katsanos, J Brynskov, and J-L Teillaud.

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