

Anti-infliximab IgE and non-IgE antibodies and induction of infusion-related severe anaphylactic reactions

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Abstract

Background: Infliximab is a chimeric monoclonal antibody against TNF- α useful in the treatment of many chronic inflammatory diseases. Severe anaphylaxis has been reported during therapy, although the exact mechanism has not been fully defined. The reactions have been related to the infliximab immunogenicity and development of specific antibodies.

Aims of the study: Evaluation of the development of IgE and non-IgE antibodies to infliximab and their relationship with infusion reaction.

Methods: Seventy-one patients (11 reactives, 11 therapeutically nonresponders, and 49 unreactive therapeutically responders) and 20 non-infliximab-exposed control subjects (ten rheumatoid arthritis, five spondyloarthropathies, five vasculitis) were evaluated for the presence of IgE (ImmunoCAP assay), IgM, and non-isotype-specific (ELISA assays) anti-infliximab antibodies. Sera were obtained at baseline and during the course of treatment, before each infliximab infusion.

Results: Eleven out of 71 patients had a hypersensitivity reaction to infliximab. Non-isotype-specific anti-infliximab antibodies were detected in eight reactive and two nonresponder patients. Three patients with severe reactions displayed anti-infliximab IgE antibodies and positive skin testing. Detectable levels of anti-infliximab IgM antibodies were shown in three additional IgE- and skin testing-negative patients. IgE and IgM antibodies to infliximab were not detectable in the two nonresponder patients. Antibodies developed before the 2nd and the 3rd infusion, and their appearance was strictly related to the timing of the reaction.

Conclusions: This report indicates that in some patients with infliximab-related severe reactions, IgE or IgM antibodies against infliximab were detectable. The majority of reactions could be predicted by the appearance of anti-infliximab antibodies.

Infliximab is a chimeric monoclonal antibody against TNF- α useful in the treatment of immuno-mediated inflammatory diseases. Anaphylaxis has been reported during this therapy, although the exact pathogenic mechanism has not been fully defined. The reactions have

been related to the infliximab immunogenicity and development of specific antibodies (Abs) (1, 2). It has been shown that antibodies to infliximab (ATI) are mainly of the IgG isotype; however, the existence of other classes of Abs has been postulated (3) but never shown.

Methods

Patients

Seventy-one consecutive infliximab-treated patients suffering from rheumatoid arthritis ($n = 23$), spondyloarthropathies ($n = 14$), and systemic vasculitis ($n = 34$) were evaluated. At enrolling, patients had never been administered with biological agents. According to the recommendations, infliximab was always associated with methotrexate in patients suffering from rheumatoid arthritis. All enrolled patients were treated with infliximab as resistant to traditional immunosuppressive therapies, including methotrexate, cyclosporine, and cyclophosphamide.

We defined reactive patients those with acute infusion reactions occurring during or within 24 h after infusion. Systemic reactions were classified as mild, moderate, or severe according to the grading system for generalized hypersensitivity reaction proposed by Brown et al. (4). Eleven patients were defined as therapeutically nonresponders because increased doses and/or shortened intervals between infusions were needed. Twenty non-infliximab-exposed subjects suffering from rheumatoid arthritis ($n = 10$), spondyloarthropathies ($n = 5$), and vasculitis ($n = 5$) were enrolled as control group. Written informed consent was provided by all patients under an institutional review board-approved protocol. Patients were infused with infliximab over a 2-h period at 0, 2, 6 weeks and every 8 weeks thereafter. Pretreatment with hydroxyzine (25 mg) and prednisone (25 mg) was routinely performed. Serum samples were taken before each infusion.

Detection of anti-infliximab antibodies

Non-isotype-specific ATI were measured by a double-capture ELISA kit (Immundiagnostick AG) according to the manufacturer's instructions. Anti-infliximab IgM Abs were measured by a modified ELISA. Samples (100 μ l of serum diluted 1 : 200) were incubated overnight (4°C) into 96-well microtiter plates precoated with infliximab (Immundiagnostick AG). After washing, biotinylated anti-human IgM (0.5 μ g/ml) (mouse IgG1k; Pharmingen, San Diego, CA, USA) was added for 1 h at RT. After 1 h of incubation with avidine peroxidase and subsequent addition of the substrate solution (TMB) for 15', the reaction was stopped with 0.4M sulfuric acid. In both assay, sera were considered to be positive when exceeded the cut off value [twice the optical density (OD) of the negative control].

Total and anti-infliximab IgE antibodies serum detection

IgE ATI were measured with the use of the streptavidin ImmunoCAP assay (kindly provided by Phadia Diagnostics, Uppsala, Sweden) as previously reported (5, 6). The threshold value for a positive result was 0.10 kUA/l. Total serum IgE detection was performed by CAP system immunoassay (Phadia Diagnostics).

Skin testing

Skin testing was carried out with the commercial infliximab preparation; the starting concentrations were 1 : 1000 for the prick test and 1 : 10 000 for the intradermal test. In both prick and intradermal testing, a minimum wheal area of 3 mm in diameter or an increase of area > 3 mm was considered as a positive response compared to a negative response to the saline control. Undiluted infliximab preparation did not show any irritating effect in non-infliximab-exposed control subjects.

Statistical analysis

A χ^2 -test was used to analyze patients groups and $P < 0.05$ value considered as significant.

Results

Fifteen acute reactions out of 1041 infusions (1.44%) were observed in 11 out of 71 patients (15.4%). Two reactions (13.4%) were classified as mild (characterized by urticaria), 8 (53.3%) as moderate (characterized by urticaria, chest tightness and dyspnea, vomiting), and 5 (33.3%) as severe because characterized by oxygen desaturation (<92%), hypotension (systolic blood pressure < 90 mmHg), and confusion (Table 1). All patients experienced one reaction, with the exception of patient number 6 who displayed recurrent (totally 5) reactions. All these five reactions were not severe, so the patient was retreated by increasing the dose of steroids during pretreatment and reducing the rate of infusion. Despite these procedures, reactions still occurred and infliximab therapy was stopped after the fifth reaction.

By using a non-isotype-specific double format ELISA test, we evaluated the presence of ATI in all treated patients (11 nonresponders, 11 reactive-, and 49 unreactive responders) and in 20 non-infliximab-exposed control subjects. Serum ATI were detected in a significantly ($P < 0.03$) higher proportion of

Table 1 Clinical data and allergological test results of patients with infusion reaction to infliximab

Patients (n)	Gender/age	Disease (n)	Grading of reaction*	Atopy/total IgE (kU/l)	SPT	Intradermal	
						15'	24 h
Reactive							
1	F/57	SpA	Severe	No/16.7	Neg	Pos	Neg
2	F/49	Vas	Moderate	No/12.3	Neg	Pos	Neg
3	M/55	RA	Severe	No/58.8	Neg	Pos	Neg
4	M/42	RA	Severe	No/25.1	Neg	Neg	Neg
5	F/46	RA	Moderate	No/15.7	Neg	Neg	Neg
6	F/37	RA	Moderate	No/68.3	Neg	Neg	Neg
7	M/41	SpA	Severe	Yes/91.2	Neg	Neg	Neg
8	F/62	RA	Moderate	No/2.8	Neg	Neg	Neg
9	M/67	Vas	Severe	No/<2.0	Neg	Neg	Neg
10	M/53	SpA	Mild	No/21.1	Neg	Neg	Neg
11	M/48	Vas	Mild	No/4.1	Neg	Neg	Neg
Unreactive (49)	F/M (29/20)	RA (15)	–	Yes/no (11/38)	0/32‡	0/32‡	0/32‡
	48 ± 9†	SpA (8)		46.5 ± 8.1†			
		Vas (26)					
Nonresponders (11)	F/M (3/8)	RA (3)	–	Yes/no (2/9)	0/11	0/11	0/11
	47 ± 5†	SpA (3)		30.2 ± 7.6†			
		Vas (5)					
Controls (20)	F/M (12/8)	RA (10)	–	Yes/no (6/14)	0/20	0/20	0/20
	51 ± 18†	SpA (5)		65.9 ± 15.2†			
		Vas (5)					

SpA, seronegative spondyloarthritis; RA, rheumatoid arthritis; Vas, vasculitis; SPT, skin prick test.

*According to Brown's classification as reported in Methods.

†Mean ± SD.

‡skin testing have been performed in a subgroup of unreactive patients.

reactive patients (8 out of 11, 72.7%) compared to nonresponders (2 out of 11, 18.1%). The ATI titer (expressed as mean OD ± SEM) was significantly higher in reactive patients than in nonresponder patients (0.76 ± 0.27 vs 0.08 ± 0.02 ; $P < 0.002$). All unreactive patients resulted ATI negative as well as non-infliximab-exposed control subjects (Fig. 1A). Unreactive patients resulted ATI negative even after many treatments (data not shown). ATI were detectable in the sera of reactive patients with severe ($n = 4$) and moderate ($n = 4$) reactions.

We investigated the hypothesis that IgE Abs against infliximab were involved in adverse reactions using an infliximab-bound CAP system. IgE ATI were detectable in 3 out of 11 (27.2%) reactive patients (Fig. 1B). To confirm the specificity of the assay, we showed that the addition of increasing doses of infliximab resulted in a significant (more than 70% with the dose of 1 µg/ml) dose-dependent reduction of specific IgE (data not shown). Because infliximab is a chimeric molecule, mouse IgG was used as control and a similar inhibition was observed. The three patients showing detectable serum ATI IgE developed a positive intradermal test at immediate lecture performed with 1/10 diluted

drug. All tested unreactive responder and controls subjects displayed negative skin testing (Table 1).

Because ATI IgE were detectable in the sera of only three out of eight ATI-positive reactive patients, the presence of IgM anti-infliximab Abs was investigated. By using a modified ELISA test, significantly higher levels of ATI IgM were found in reactive patients (0.86 ± 0.24) compared to unreactive subjects (0.18 ± 0.03) and controls (0.25 ± 0.06 ; $P < 0.02$ and $P < 0.05$, respectively). In particular, high levels of IgM ATI were found in three IgE ATI-negative patients (Fig. 1C and Table 2).

IgE and IgM ATI were not detectable in the two nonresponder ATI-positive patients.

Finally, we asked whether a time relationship between the ATI appearance and the infliximab reaction could be shown. The detection of ATI in serum collected before the first infliximab infusion resulted negative in all subjects. Antibodies were detected in the sera collected before the 2nd and the 3rd infusion, and their appearance was strictly related to the timing of the reaction (Table 2). Monitoring the ATI titer, a progressive but quite fast decrease of ATI (IgE included), starting from 7 days after the reaction was observed (Fig. 1D).

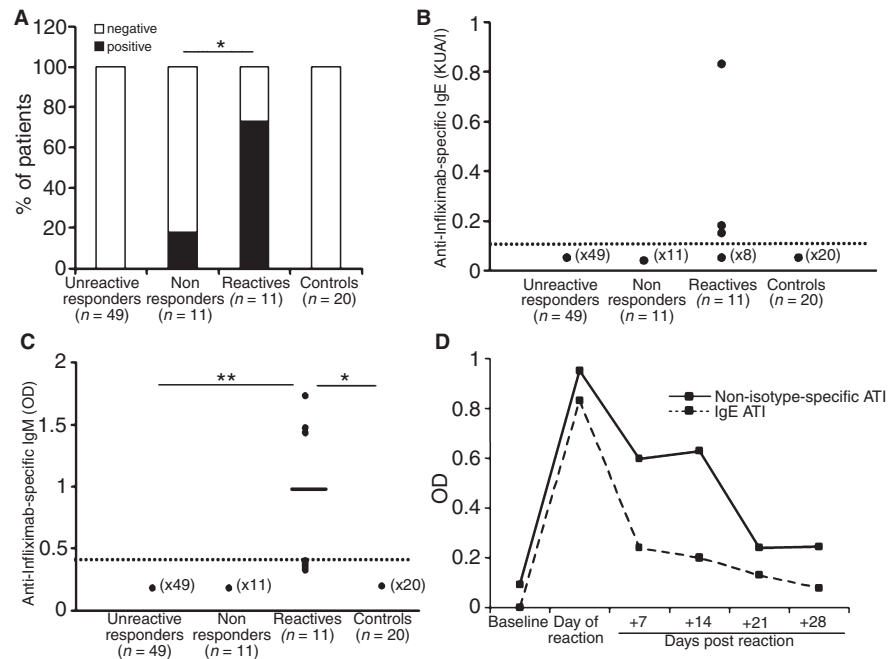


Figure 1 Incidence, isotype and kinetic of serum anti-infliximab antibodies. (A) Proportion (black columns) of patients displaying serum anti-infliximab antibodies (ATI), measured with a non isotype-specific ELISA test; * $P < 0.05$. (B) ATI IgE detection using ImmunoCAP assay is indicated. The dotted line represents the cut-off value for positive results (0.10 KUA/l). (C) Serum titer of ATI IgM in different groups of patients and controls. Values are reported as optical density (OD). The horizontal line indicates geometric mean values; the dotted line represents cut-off value (OD = 0.4); ** $P < 0.02$. (D) Longitudinal analysis of non isotype-specific (black line) and IgE (dotted line) ATI in the serum of a representative reactive patient (pt#1). Serum samples were obtained at baseline, the day of reaction before the infusion and 7, 14, 21 and 28 days after the reaction.

Table 2 Timing of appearance and isotype of ATI in reactive and nonresponder patients

Patients	Grading of reaction	ATI appearance*	Infusion inducing reaction	Non-isotype-specific ATI	ATI isotype	
					IgM	IgE
Reactives						
1	Severe	2nd†	2nd†	+‡	Neg	+
2	Moderate	2nd	2nd	+	Neg	+
3	Severe	2nd†	2nd†	+‡	Neg	+
4	Severe	3rd	3rd	+‡	+	Neg
5	Moderate	3rd†	3rd†	+	+	Neg
6	Moderate	ND	3rd, 4th, 5th, 7th, 9th	+‡	+	Neg
7	Severe	3rd	3rd	+	Neg	Neg
8	Moderate	2nd	4th	+	Neg	Neg
9	Severe	–	5th	Neg	Neg	Neg
10	Mild	–	9th	Neg	Neg	Neg
11	Mild	–	21th	Neg	Neg	Neg
Non responders						
1	–	14th	–	+	Neg	Neg
2	–	9th	–	+	Neg	Neg

ND, not determinable, serum negative at baseline, positive before the 7th infusion; intermediate serum samples not available; ATI, antibodies to infliximab.

*infliximab infusion before that ATI serum positivity occurred.

†second course of therapy.

‡patients displaying high serum titer (optical density > 0.9).

Of note, two out of the three patients developed reactions during the second infusion in the second course of treatment, after a period of therapy interruption.

Discussion

We analyzed the humoral immune response to infliximab, by using a combination of different assays, able to detect non-isotype-specific, IgE, and IgM anti-infliximab Abs. We totally observed ATI development in 10 out of 71 infliximab-treated patients, the majority of them ($n = 8$) being patients showing infusion reactions, whereas two positive sera were found in the group of nonresponder subjects. Our results show that a proportion of infliximab-related reactions were associated with the appearance of IgE ATI. To our knowledge, this is the first report showing serum IgE ATI and their strict relationship to acute severe reactions. The specificity of these Abs has been directed toward the murine part of the chimeric biological agent as demonstrated by the inhibition assays performed both with the drug and the mouse Ig. The low titer of IgE ATI observed, may be because of the intrinsic characteristics of the infliximab and/or to the time-limited exposure of our patients. Different than the results obtained by Chung on cetuximab (5), we excluded pre-existing sensitization in our patients. The progressive decrease of ATI IgE after the discontinuation of therapy is in agreement with this. Because a hypersensitivity reaction was suspected, skin tests (prick and i.d.) with the biological agent were performed. In our experience, the pathogenetic role of IgE ATI is supported by the positive results of infliximab skin testing. Of note, skin tests for monoclonal antibodies have been performed in few case series of patients with infusion-related reaction (3, 7), and they obviously need to be performed on much more cases for standardization.

Some data suggest that immunological mechanisms other than type I hypersensitivity may be involved in protein-induced fatal anaphylaxis, at least in animal models (8, 9). We demonstrate that the presence of IgM ATI is involved in severe reaction. In two ATI-positive but IgE- and IgM-negative patients, a role for anti-drug IgG isotype may be envisaged. Of note, two out of three patients with adverse reactions and no detectable ATI of all isotypes did not show severe symptoms. At present, the exact mechanisms involved in these patients remain unclear.

Finally, this study defines a timing relationship between the ATI appearance and the infliximab reaction. It is interesting to note that in three patients,

ATI were detectable at the second course of treatment after the first infusion suggesting a sort of sensitization phase (first course of treatment) and a challenge phase (second course of treatment). This agrees with previous studies describing an effect of therapy discontinuation on the prevalence of infliximab reaction (10).

This report clearly indicates that the majority of reactions could be predicted by the appearance of anti-infliximab Abs. By monitoring these Abs, at least for the first five infusions, it is possible to identify potentially reactive patients and, thus, improve the infliximab safety profile. The availability of an assay able to detect ATI IgE Abs appears to have significant relevance from a clinical point of view, taking into account the increasing utilization of biological drugs.

References

1. Cheifetz A, Mayer L. Monoclonal antibodies, immunogenicity and associated infusion reactions. *Mt Sinai J Med* 2005;**72**:250–256.
2. Baert F, Noman M, Vermeire S, Van Assche GD, Haens G, Carbonez A et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003;**348**:601–608.
3. Pichler WJ. Adverse side-effects to biological agents. *Allergy* 2006;**61**:912–920.
4. Brown SG. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol* 2004;**114**:371–376.
5. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M et al. Cetuximab-induced anaphylaxis and IgE specific for galactose- α -1,3-galactose. *New Engl J Med* 2008;**358**:1109–1117.
6. Erwin EA, Custis NJ, Satinover SM, Perzanowski MS, Woodfolk J, Crane J et al. Quantitative measurement of IgE antibodies to purified allergens using streptavidin linked to a high-capacity solid phase. *J Allergy Clin Immunol* 2005;**115**:1029–1035.
7. Campi P, Benucci M, Manfredi M, Demoly P. Hypersensitivity reactions to biological agents with special emphasis on tumor necrosis factor- α antagonists. *Curr Opin Allergy Clin Immunol* 2007;**7**:393–403.
8. Finkelman FD. Anaphylaxis: lessons from mouse models. *J Allergy Clin Immunol* 2007;**120**:506–515.
9. Oettgen HC, Martin TR, Wynshaw-Boris A, Deng C, Drazen JM, Leder P. Active anaphylaxis in IgE-deficient mice. *Nature* 1994;**370**:367–370.
10. Vultaggio A, Matucci A, Parronchi P, Rossi O, Palandri F, Romagnani S et al. Safety and tolerability of infliximab therapy: suggestions and criticisms based on wide clinical experience. *Int J Immunopathol Pharmacol* 2008;**21**:367–374.