

CONCISE REPORT

Infusion reactions during infliximab treatment are not associated with IgE anti-infliximab antibodies

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ABSTRACT

Objectives Controversy exists on the role of IgE antidrug antibodies (IgE-ADA) in infusion reactions (IR) on infliximab treatment, partly due to the lack of a positive control used for assay validation. We sought to (1) develop a robust assay to measure IgE-ADA, including a positive control, (2) determine the association between IgE-ADA and IR and (3) determine the incidence of IgE-ADA in infliximab treated patients.

Methods A recombinant human IgE anti-infliximab monoclonal antibody was developed as standard and positive control. With this antibody, we set up a novel robust assay to measure IgE-ADA. IgE-ADA was determined in three retrospective cohorts (n=159) containing IR+ (n=37) and IR- (n=39), and longitudinal sera of 83 spondyloarthritis.

Results IgE-ADA was found in 0/39 IR-, whereas 4/37 (11%) IR+ showed low levels (0.1–0.3 IU/mL, below the 0.35 IU/mL threshold associated with elevated risk of allergic symptoms). All patients who were IgE-ADA positive also had (very) high IgG-ADA levels. The incidence of IgE-ADA in patients with infliximab-treated spondyloarthritis was estimated at less than approximately 1%.

Conclusions IgE-ADA is rarely detected in infliximab-treated patients. Moreover, the absence of IgE-ADA in the majority of IR+ patients suggests that IgE-ADA is not associated with infusion reactions.

INTRODUCTION

During treatment of inflammatory conditions with infliximab, some patients experience adverse events called infusion reactions (IR). The reported incidence of IR varies between studies, ranging from 4% to 15% across both early and more recent trials.^{1–7} With the infliximab biosimilar CT-P13, a comparable incidence of IR to reference infliximab has been reported, averaging around 12%.^{3,5}

IR are mostly mild to moderate, with the most common symptoms being pruritus, flushing and dyspnoea.⁶ In rare cases, severe IR are described with symptoms such as hypotension, respiratory distress, chest tightness and other anaphylactoid reactions.^{8,9} Since these symptoms resemble those of a Type I allergic reaction and occur on administration of infliximab, IR have been suggested to be mediated by IgE antidrug antibodies (IgE-ADA). Indeed, several studies show detection of IgE-ADA in part of the patients with IR, although in the study by Benucci *et al* IgE-ADA was also detected in several IR- patients.^{10–12}

Measurement of IgE-ADA has thus far been performed with assays that were not designed to overcome specific therapeutic antibody-related issues, including underestimation of IgE-ADA by high drug levels or rheumatoid factor (RF) causing false positivity due to cross-linking of, for example, coating and detecting antibodies. Importantly, specific positive controls were lacking in these assays, hampering assay development and interpretation.

We here describe a novel assay to measure IgE-ADA that circumvents these issues and includes a recombinant human monoclonal IgE anti-infliximab antibody as calibrator. We investigated the association of IgE-ADA with infusion reactions and determined its general incidence in patients with spondyloarthritis.

MATERIALS AND METHODS

See online supplementary methods.

RESULTS

Construction of human IgE anti-infliximab

For construction of a positive control and calibration curve, a human IgE anti-infliximab clone was developed. From two patients with high IgG-ADA titres, eight human monoclonal IgG anti-infliximab antibodies were obtained by sequencing the variable heavy (VH) and variable light (VL) of individual infliximab-specific B cells (see online supplementary table 1). One clone, mAb 1.1, was recombinantly expressed as IgE. Infliximab specificity and IgE isotype were confirmed on blot (figure 1A).

Development of an IgE-ADA assay

We developed an IgE-ADA assay as an antigen-binding test: capture of total IgE from a sample followed by detection of specific IgE using biotinylated infliximab (figure 1B). Since infliximab is an IgG antibody, and antibodies are used as a reagent for IgE capture, we envisaged potential background issues due to RF and antihinge activity.¹³ Furthermore, binding of IgG4-ADA via Fc interactions might also cause false-positive signals.¹⁴ This was remedied by (1) using F(ab')₂ fragments of both capture antibody and infliximab to remove RF backgrounds, (2) addition of intravenous immunoglobulin F(ab')₂ to remove antihinge background, and (3) addition of irrelevant IgG4 to block binding of IgG4-ADA. A representative calibration curve is



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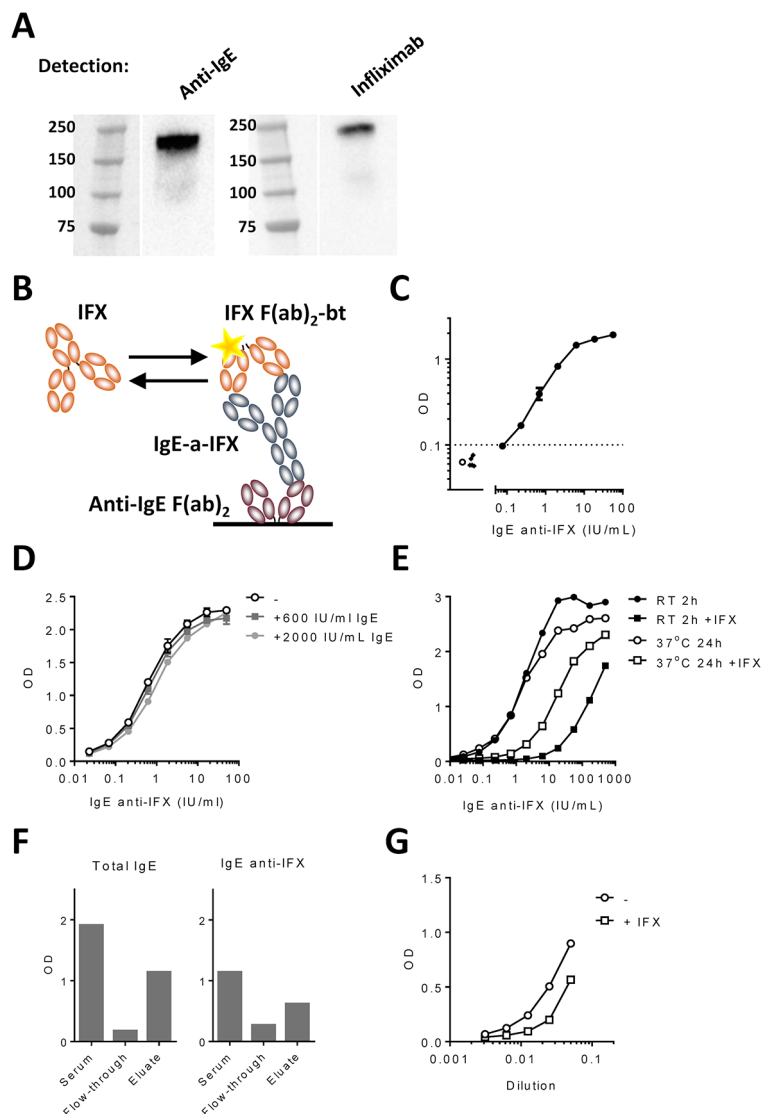


Figure 1 ELISA for measurement of IgE antidrug antibodies (IgE-ADA). (A) Western blots of IgE anti-infliximab. Anti-IgE (left) and infliximab (IFX, right) are used as detection antibodies. The molecular weight of IgE is approximately 190 kDa. (B) Anti-IgE F(ab')₂ is used to capture IgE, followed by detection using biotinylated infliximab F(ab')₂. To enhance drug-tolerance, the second step involves overnight incubation at 37°C. (C) Calibration curve of the recombinant IgE anti-infliximab 1.1 mAb. Dotted line indicates the detection limit, open circle indicates blank, and black diamonds are randomly selected serum samples containing rheumatoid factor and/or antihinge antibodies. Samples are diluted 1:10 and units on x-axis represent those in undiluted sample throughout. (D) Calibration curve with and without additional irrelevant polyclonal IgE (600 or 2000 IU/mL). (E) Calibration curve in the absence or presence of infliximab (100 µg/mL), on incubation with biotinylated infliximab F(ab')₂ for either 2 hours at room temperature (RT, closed symbols) or overnight at 37°C (open symbols). Drug tolerance is enhanced by prolonged incubation at elevated temperature; the second protocol was used for all other experiments. (F) A serum containing a high titre of IgE-ADA was depleted for total IgE using an anti-IgE Sepharose column, and flow-through and eluate were analysed for total IgE (left panel) and IgE-ADA (right panel). (G) This serum, which contained no detectable infliximab, was also used to assess drug tolerance of the ELISA by adding 100 µg/mL infliximab.

shown in [figure 1C](#) together with blank and RF+/anti-hinge+ control sera.

Potential loss of sensitivity due to non-specific IgE in serum samples was investigated by adding high amounts of polyclonal serum-derived IgE to the calibrator. Only the highest concentration resulted in a modest (<1.5-fold) reduction in signal ([figure 1D](#)). Less than approximately 5% of patients with rheumatoid arthritis will have total IgE levels approaching this value, effectively ruling out interference from total IgE.¹⁵

Drug tolerance of our initial protocol (2-hour incubation with biotinylated infliximab F(ab')₂ at room temperature) was poor: a large excess of infliximab reduced the signal from the calibrator 100-fold ([figure 1E](#)). Overnight incubation at 37°C resulted in

an improved, modestly drug-tolerant assay, with a nearly 10-fold reduction in signal in the presence of 100 µg/mL of infliximab ([figure 1E](#)), which was unaffected by the additional presence of IgG-ADA (not shown). The technical cut-off was 0.08 IU/mL and values >0.1 IU/mL were considered positive. The cut-off of 0.35 IU/mL was used to determine clinical relevance, since values above this threshold are associated with elevated risk of allergic symptoms.¹⁶

With the IgE-ADA assay, we determined the frequency of IgE-ADA in IgG-ADA positive samples. Sera of 54 patients treated with infliximab sent to our diagnostics department were selected based on their IgG-ADA levels (median 1800 AU/mL; range: 510–140 000). Fifty-three sera were negative for

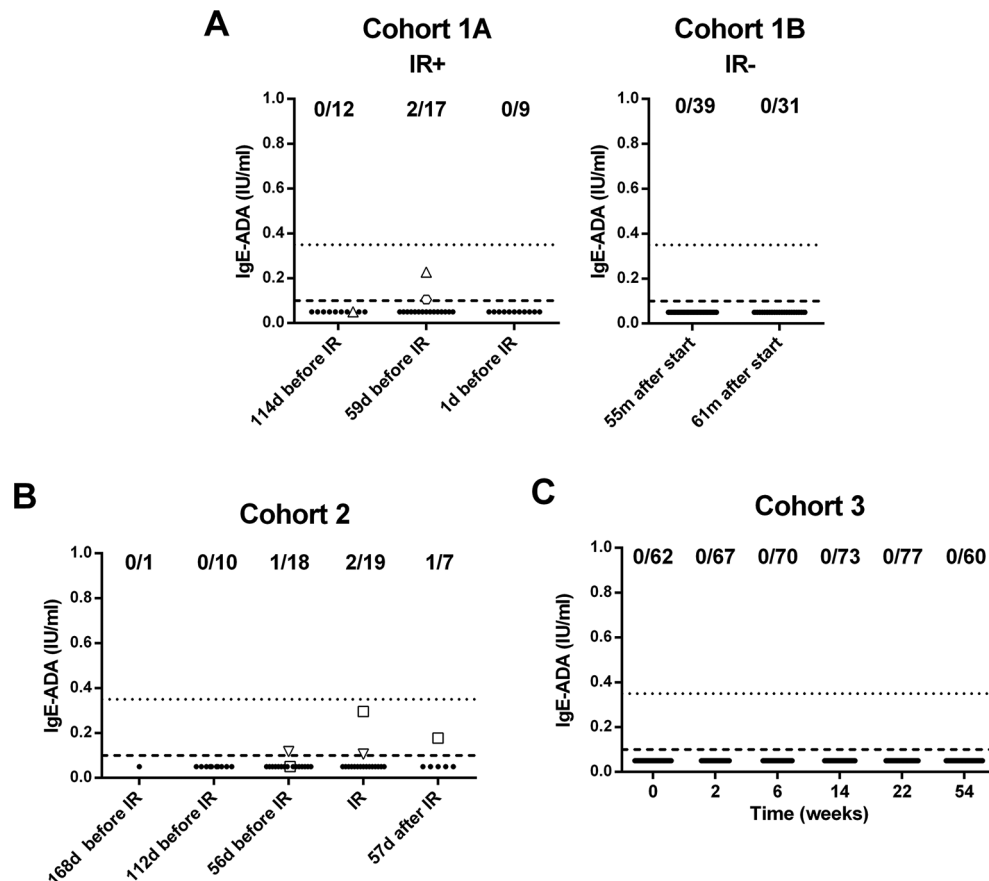


Figure 2. IgE antidrug antibody (IgE-ADA) levels determined in patient sera. (A) Cohort 1A consisted of infusion reaction (IR)+ patients that were either IgG-ADA+ or IgG-ADA- (n=19, left). All samples measured at median 114 days before IR have a paired sample at median 59 days before IR. Cohort 1B consisted of IR- patients that were IgG-ADA+ in the bridging ELISA (n=39, right). (B) Cohort 2 consisted of IR+ patients that were either IgG-ADA+ or IgG-ADA- (n=18). (C) Cohort 3 consisted of longitudinal samples of IgG-ADA+ or IgG-ADA- patients (n=83). Patients that were IgE-ADA positive in at least one time point are depicted by open symbols. Numbers stated above each time point indicate: number of IgE-ADA positive samples/all measured samples. Indicated time in A and B is the median, m=months, d=days. Dashed line = detection limit, dotted line = cut-off for clinical relevance.¹⁶

IgE-ADA, whereas one serum was positive (28 IU/mL). This latter sample contained no detectable infliximab and a very high titre of IgG-ADA (140 000 AU/mL).

To assess the specificity of the assay, the serum was depleted for IgE, resulting in removal of specific signal alongside reduction of total IgE (figure 1F), confirming the specificity. Drug tolerance was also investigated using this sample: adding 100 µg/mL infliximab to the sample prior to analysis reduced the signals approximately threefold to fourfold (figure 1G).

Association of IgE-ADA with IR

We investigated the association of IgE-ADA in relation to IR. To this end, patients in cohort 1A (n=19) and cohort 2 (n=18) were selected on presence of an IR (see online supplementary table 3 for symptoms), irrespective of IgG-ADA status. As a comparison cohort 1B (n=39) was used, containing IR-negative patients from the same centre as cohort 1A, but selected based on ADA positivity, measured by bridging ELISA.

Of cohort 1A, samples taken at median 1 (IQR 1–3), 59 (IQR 51–75) and 114 (IQR 92–119) days prior to IR were analysed. Detectable IgE-ADA levels were found in 2/19 patients (0.10 and 0.23 IU/mL) at time-point 59 days prior to IR; 17/19 patients were negative for IgE-ADA on all time points (figure 2A). Of cohort 2, samples taken several time points before and after IR, and just prior to IR were analysed (figure 2B). Low IgE-ADA

levels were detected in 2/18 patients just before IR (0.30 and 0.11 IU/mL) and either 8 weeks before (0.12 IU/mL) or after IR (0.18 IU/mL). Sixteen patients did not have detectable IgE-ADA. Of cohort 1B, no IgE-ADA was detected in any of the samples taken median 55 (IQR 25–100) and 61 (IQR 33–102) months after start of treatment (figure 2A).

Incidence of IgE-ADA

We investigated the general IgE-ADA incidence in infliximab-treated patients, as well as the possibility of an early IgE-ADA response shortly after infliximab treatment, using a retrospective longitudinal cohort of 83 patients with spondyloarthritis that were followed for 52 weeks (cohort 3). However, no IgE-ADA was detected in any of the samples, suggesting an incidence of less than approximately 1% in patients with infliximab-treated spondyloarthritis (figure 2C).

DISCUSSION

The role of IgE-ADA in infusion reactions is under debate. In this study, we developed a robust assay to measure IgE-ADA and created a recombinant human IgE anti-infliximab antibody to use as standard. IgE-ADA was detected in 5/213 infliximab-treated patients, which in only one case exceeded the cut-off of 0.35 IU/mL. In IR+ patients, 4/37 (11%) had detectable IgE-ADA levels,

indicating that the majority of infusion reactions are not related to IgE-ADA.

Previous studies reported frequencies of 1/5 and 6/30 in IR+ patients.^{10 12} However, comparison with these studies is troublesome. For instance, the assays used are generally not optimised to measure IgE specific to therapeutic antibodies. Furthermore, comparing frequencies between studies is hampered by the ill-defined definition of an infusion reaction and its associated classification system of mild, moderate and severe reactions.

Four out of five IgE-ADA-positive patients had documented infusion reactions; clinical data of the fifth patient was not known. All patients also had high levels of IgG-ADA (an estimated >1000-fold excess) which may have competed with IgE-ADA for infliximab, thereby ameliorating IgE driven effects. The study by Cheifetz *et al* showing normal tryptase levels in IR+ patients supports the notion that these reactions are not IgE mediated.¹⁷

Nevertheless, in case of infliximab as the 'allergen', IgG-ADA may alternatively induce type III hypersensitivity. Thus far, the only factor correlating with IR is the (IgG or total immunoglobulin) ADA titre: IR+ patients have a significantly higher ADA titre than patients without adverse events.⁷ The mechanism behind the clinical effects is thought to be immune complex formation between infliximab and (IgG) ADA.¹⁸

Importantly, not all patients with a high ADA titre experience IR, and IR are also observed in patients without detectable ADA, indicating that factors other than ADA can be involved.

In the longitudinal retrospective cohort, none of the samples were positive for IgE-ADA during the first year, whereas in 13/83 patients IgG-ADA were detected. The absence of IgE-ADA in these patients suggests that the general incidence of IgE antibody formation is low.

The IgE-ADA assay described in this study is, besides its RF tolerance, also moderately drug tolerant. It is however possible that, through complex formation with infliximab or due to low affinity, very small amounts of IgE-ADA will not be detected. Moreover, in cohort 1A, part of the samples of IR+ patients taken shortly before IR are missing, and the possibility exists that IgE-ADA was developed after median 59 days.

In short, this study shows a frequency of 11% in IR+ rheumatic patients, and an incidence of less than approximately 1% in patients with infliximab-treated spondyloarthritis. IgE-ADA was generally found in very low levels, and all IgE-ADA-positive patients were also IgG-ADA positive. To the extent that ADA play a role in eliciting an IR, we expect IgG to be a more dominant risk factor than IgE.

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