## Immunogenicity of therapeutic antibodies

*Immunological mechanisms & clinical consequences*

van Schie, K.A.J.

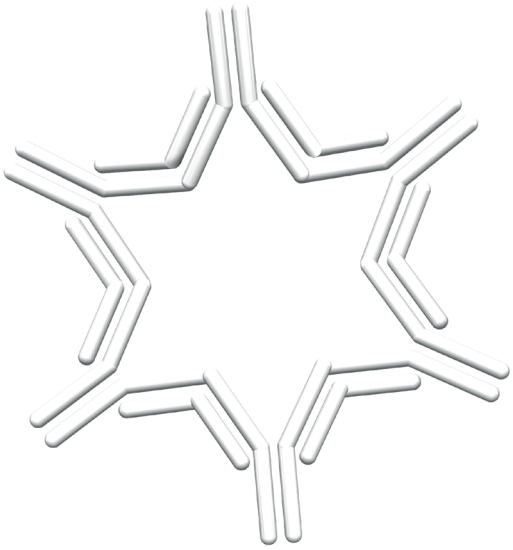
**Publication date**
2017
**Document Version**
Other version
**License**
Other

# Chapter 6

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

Ann Rheum Dis. 2017;76:1285-1288

Karin A. van Schie, Pleuni Ooijevaar-de Heer, Simone Kruithof, Chamaida Plasencia, Teresa Jurado, Dora Pascual-Salcedo, Johannan F. Brandse, Geert R.A.M. d'Haens, Gerrit Jan Wolbink & Theo Rispens



# ABSTRACT

**Objectives:** Controversy exists on the role of IgE anti-drug antibodies (IgE-ADA) in infusion reactions (IR) upon infliximab treatment, partly due to the lack of a positive control used for assay validation. We sought to I) develop a robust assay to measure IgE-ADA, including a positive control, II) determine the association between IgE-ADA and IR, and III) 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) patients, and longitudinal sera of 83 spondyloarthritis patients.

**Results:** IgE-ADA was found in 0/39 IR- patients, whereas 4/37 (11%) IR+ patients showed low levels (0.1-0.3 IU/ml, below the 0.35 IU/mL threshold associated with elevated risk of allergic symptoms). All IgE-ADA positive patients also had (very) high IgG-ADA levels. The incidence of IgE-ADA in infliximab treated spondyloarthritis patients 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 dyspnea.[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 upon administration of infliximab, IR have been suggested to be mediated by IgE-ADA. Indeed, several studies show detection of IgE-ADA in part of the IR+ patients, 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 crosslinking 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 spondyloarthritis patients.



# MATERIALS AND METHODS

Methods are described in 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 titers, eight human monoclonal IgG anti-infliximab antibodies were obtained by sequencing the VH and VL

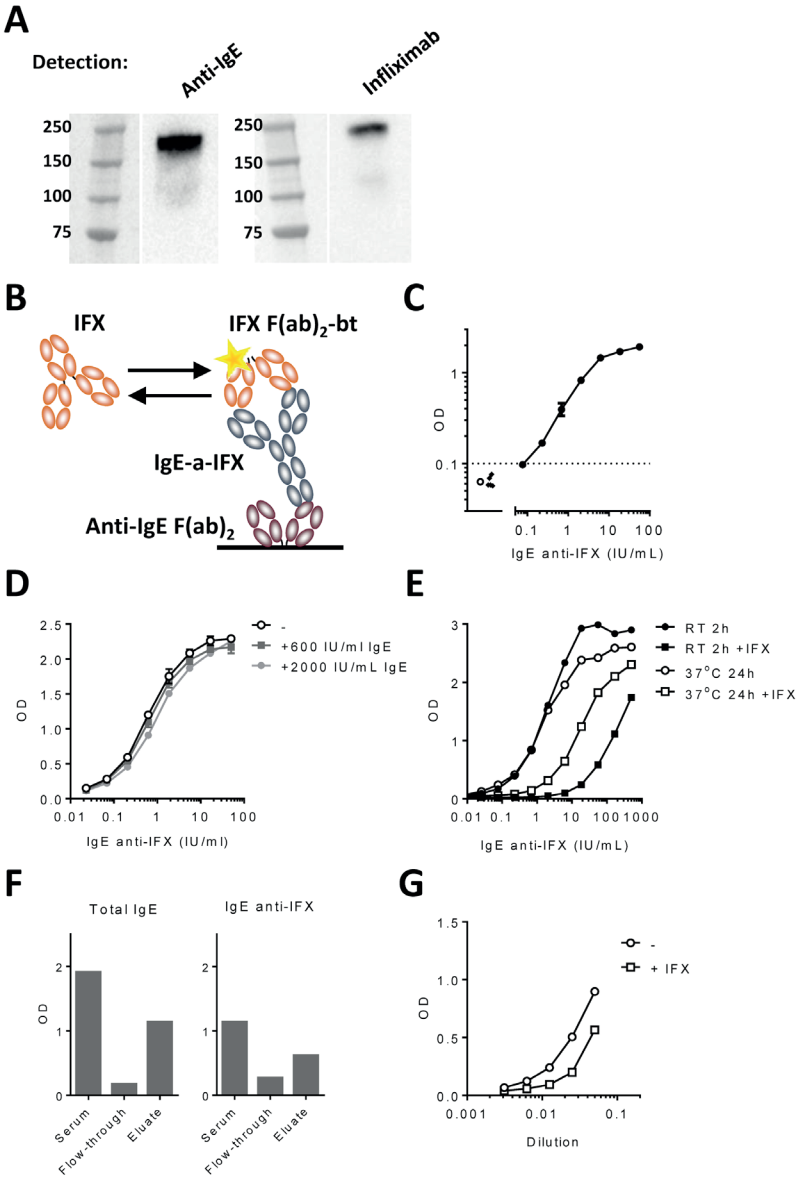



**Figure 1.** ELISA for measurement of IgE-ADA. **A)** Western blots of IgE anti-infliximab. Anti-IgE (left) and infliximab (IFX, right) are used as detection antibodies. The molecular weight of IgE is approximately 190 kDa. **B)** Anti-IgE F(ab')$_2$ is used to capture IgE, followed by detection using 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 anti-hinge 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).

**Figure 1.** ELISA for measurement of IgE-ADA. (continued) **E)** Calibration curve in the absence or presence of infliximab (100 µg/ml), upon 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 titer 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.

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 anti-hinge activity.[13] Furthermore, binding of IgG4-ADA via Fc interactions might also cause false-positive signals.[14] This was remedied by I) using F(ab')$_2$ fragments of both capture antibody and infliximab to remove RF backgrounds, II) addition of IVIG F(ab')$_2$ to remove anti-hinge background, and III) addition of irrelevant IgG4 to block binding of IgG4-ADA. A representative calibration curve is 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 RA patients will have total IgE levels approaching this value, effectively ruling out interference from total IgE.[15]

Drug tolerance of our initial protocol (2hr 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 infliximab treated patients 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 IgE-ADA, whereas one serum was positive (28 IU/ml). This latter sample contained no detectable infliximab and a very high titer 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 3-4-fold (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 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 eight 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 spondyloarthritis patients 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 infliximab treated spondyloarthritis patients (Figure 2C).

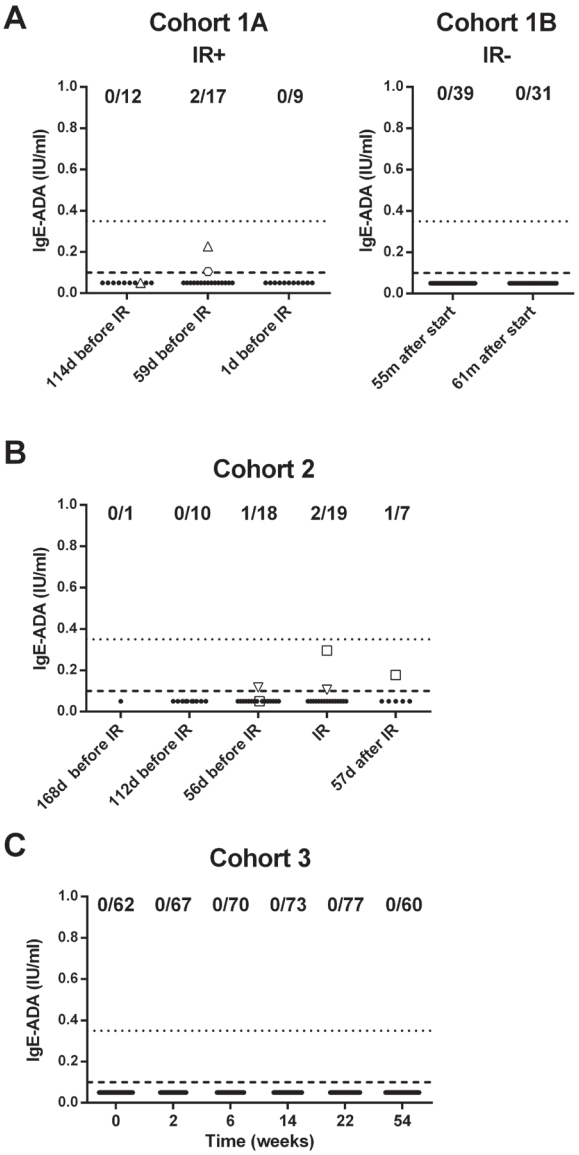



**Figure 2.** IgE-ADA levels determined in patient sera. **A**) Cohort 1A consisted of IR+ patients that were either IgG-ADA+ or IgG-ADA- (n=19, left). All samples measured at median 114 days before IR have a paired sample at median 59 days before IR. Cohort 1B consisted of IR- patients that were IgG-ADA+ in the bridging ELISA (n=39, right). **B**) Cohort 2, IR+ patients that were either IgG-ADA+ or IgG-ADA- (n=18). **C**) Cohort 3, 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]

# 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 optimized 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 Ig) ADA titer: IR+ patients have a significantly higher ADA titer 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 titer experience an 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 13/83 patients were IgG-ADA positive. 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 infliximab treated spondyloarthritis patients. 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.

**Acknowledgements**

We are very grateful to Annick de Vries and Henk de Vrieze for their valuable contributions to this project. We thank Rob Aalberse for his valuable suggestions and his critical revision of the manuscript. Funding of this study was provided by an unrestricted grant from Pfizer. Pfizer had no involvement in the study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

**Conflict of interest**

K.A. van Schie, S. Kruithof and C. Plasencia-Rodrigues report grants from Pfizer during the conduct of the study. J.F. Brandse reports speaker fees from: Merck Sharp & Dohme, Abbvie and Takeda pharmaceuticals, outside the submitted work. G.R.A.M. d'Haens reports grants from AbbVie, Janssen, Given Imaging, MSD, Dr Falk Pharma, PhotoPill, consulting and/or lecture fees from Abbvie, ActoGeniX, AIM, Boehringer Ingelheim GmbH, Centocor, ChemoCentryx, Cosmo Technologies, Elan Pharmaceuticals, enGene, Dr Falk Pharma, Ferring, Galapagos, Giuliani SpA, Given Imaging, GlaxoSmithKline, Janssen Biologics, MSD, Neovacs, Novo Nordisk, Otsuka, PDL BioPharma, Pfizer, Receptos, Salix, SetPoint, Shire Pharmaceuticals, Schering-Plough, Takeda, Tillotts Pharma, UCB Pharma, Versant, Vifor Pharma, and speaking honoraria from AbbVie, Tillotts, Tramedico, Ferring, MSD, UCB Pharma, Norgine, Shire, outside the submitted work. G. Wolbink reports grants from Pfizer during the conduct of the study; grants from Pfizer, payment for lectures from Pfizer, Abbvie and UCB outside the submitted work. T. Rispens reports grants from Pfizer during the conduct of the study; grants from Genmab, consultancy fees from Genmab and payment for lectures from Pfizer, Abbvie and Regeneron outside the submitted work. P. Ooijevaar-de Heer, T. Jurado and D. Pascual-Salcedo have nothing to disclose.

# REFERENCES


1. Ducourau E, Mulleman D, Paintaud G, et al. Antibodies toward infliximab are associated with low infliximab concentration at treatment initiation and poor infliximab maintenance in rheumatic diseases. *Arthritis Res Ther*. 2011;13(3):R105. doi:10.1186/ar3386.
2. Maini R, St Clair EW, Breedveld F, et al. Infliximab (chimeric anti-tumour necrosis factor α monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. *Lancet*. 1999;354(9194):1932-1939. doi:10.1016/S0140-6736(99)05246-0.
3. Park W, Yoo DH, Jaworski J, et al. Comparable long-term efficacy, as assessed by patient-reported outcomes, safety and pharmacokinetics, of CT-P13 and reference infliximab in patients with ankylosing spondylitis: 54-week results from the randomized, parallel-group PLANETAS study. *Arthritis Res Ther*. 2016;18:25. doi:10.1186/s13075-016-0930-4.
4. Shergy WJ, Isern RA, Cooley DA, et al. Open label study to assess infliximab safety and timing of onset of clinical benefit among patients with rheumatoid arthritis. *J Rheumatol*. 2002;29(4):667-677.
5. Yoo DH, Racewicz A, Brzezicki J, et al. A phase III randomized study to evaluate the efficacy and safety of CT-P13 compared with reference infliximab in patients with active rheumatoid arthritis: 54-week results from the PLANETRA study. *Arthritis Res Ther*. 2015;18(1):82. doi:10.1186/s13075-016-0981-6.
6. Choquette D, Faraawi R, Chow A, Rodrigues J, Bensen WJ, Nantel F. Incidence and management of infusion reactions to infliximab in a prospective real-world community registry. *J Rheumatol*. 2015;42(7):1105-1111. doi:10.3899/jrheum.140538.
7. Pascual-Salcedo D, Plasencia C, Ramiro S, et al. Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis. *Rheumatology (Oxford)*. 2011;50(8):1445-1452. doi:10.1093/rheumatology/ker124.
8. Steenholdt C, Svenson M, Bendtzen K, Thomsen OØ, Brynskov J, Ainsworth MA. Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2011;34:51-58.
9. Steenholdt C, Svenson M, Bendtzen K, Thomsen OØ, Brynskov J, Ainsworth MA. Acute and delayed hypersensitivity reactions to infliximab and adalimumab in a patient with Crohn ' s disease. *J Crohn's Colitis*. 2012;6(1):108-111. doi:10.1016/j.crohns.2011.08.001.
10. Matucci A, Pratesi S, Petroni G, et al. Allergological in vitro and in vivo evaluation of patients with hypersensitivity reactions to infliximab. *Clin Exp Allergy*. 2013;43(6):659-664. doi:10.1111/cea.12098.
11. Fréling E, Peyrin-Biroulet L, Poreaux C, et al. IgE antibodies and skin tests in immediate hypersensitivity reactions to infliximab in inflammatory bowel disease: impact on infliximab retreatment. *Eur J Gastroenterol Hepatol*. 2015;27(10):1200-1208. doi:10.1097/MEG.0000000000000436.
12. Benucci M, Manfredi M, Saviola G, Baiardi P, Campi P. Correlation between atopy and hypersensitivity reactions during therapy with three different TNF-alpha blocking agents in rheumatoid arthritis. *Clin Exp Rheumatol*. 2009;27:333-336.

13. van Schie KA, Wolbink G-J, Rispens T. Cross-reactive and pre-existing antibodies to therapeutic antibodies - Effects on treatment and immunogenicity. *MAbs*. 2015;7(4):662-671. doi: 10.1080/19420862.2015.1048411.

14. Rispens T, Ooievaar-De Heer P, Vermeulen E, Schuurman J, van der Neut Kolfschoten M, Aalberse RC. Human IgG4 Binds to IgG4 and Conformationally Altered IgG1 via Fc-Fc Interactions. *J Immunol*. 2009;182(7):4275-4281. doi:10.4049/jimmunol.0804338.

15. Hunder GG, Gleich GJ. Immunoglobulin E (IgE) levels in serum and synovial fluid in rheumatoid arthritis. *Arthritis Rheum*. 17(6):955-963.

16. van Hoeyveld E, Nickmans S, Ceuppens JL, Bossuyt X. Defining thresholds of specific IgE levels to grass pollen and birch pollen allergens improves clinical interpretation. *Clin Chim Acta*. 2015; 450:46-50. doi:10.1016/j.cca.2015.07.023.

17. Cheifetz A, Smedley M, Martin S, et al. The Incidence and Management of Infusion Reactions to Infliximab : A Large Center Experience. *Am J Gastroenterol*. 2003;98(6):1315-1324. doi: 10.1016/S0002-9270(03)00231-4.

18. van der Laken CJ, Voskuyl AE, Roos JC, et al. Imaging and serum analysis of immune complex formation of radiolabelled infliximab and anti-infliximab in responders and non-responders to therapy for rheumatoid arthritis. *Ann Rheum Dis*. 2007;66:253-256. doi:10.1136/ard.2006.057406.

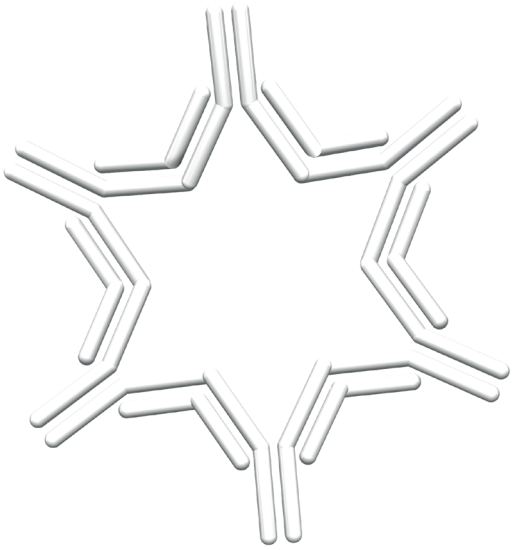
# Chapter 6

**Supplemental material**

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

Ann Rheum Dis. 2017;76:1285-1288

Karin A. van Schie, Pleuni Ooijevaar-de Heer, Simone Kruithof, Chamaida Plasencia, Teresa Jurado, Dora Pascual-Salcedo, Johannan F. Brandse, Geert R.A.M. d'Haens, Jan Wolbink & Theo Rispens



**Supplementary Table 1.** Patient-derived anti-infliximab monoclonal antibodies. For each clone, isotype, V(D)J usage, number of mutations leading to amino acid replacements (R) or silent mutations (S) and κ/λ is described. Sequences were aligned using IMGT/V-QUEST database.[1] Variable domain sequences of monoclonal anti-infliximab 1.1 were used to develop the IgE anti-infliximab calibration curve.

| Clone | Heavy chain | | | | | | Light chain | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Isotype | V gene | D gene | J gene | R | S | κ/λ | V gene | J gene | R | S |
| 1.1 | N.D. | 1-46 | 3-09 | 4 | 25 | 7 | κ | 4-1 | 2 | 8 | 4 |
| 1.2 | IgG1 | 4-34 | 5-05 | 4 | 10 | 3 | κ | 1-5 | 1 | 0 | 0 |
| 1.3 | IgG1 | 1-18 | 2-15 | 3 | 11 | 3 | λ | 2-23 | 3 | 9 | 8 |
| 1.4 | IgG4 | 1-18 | 2-15 | 4 | 11 | 5 | λ | 2-23 | 3 | 10 | 7 |
| 2.1 | IgG1 | 1-69 | 2-02 | 4 | 8 | 4 | λ | 4-69 | 2 | 11 | 7 |
| 2.2 | IgG4 | 3-74 | 3-10 | 3 | 17 | 6 | κ | 3-11 | 4 | 16 | 6 |
| 2.3 | IgG4 | 3-30 | 5-05 | 4 | 21 | 4 | κ | 1-9 | 4 | 12 | 7 |
| 2.4 | IgG4 | 3-74 | 3-10 | 3 | 20 | 10 | κ | 3-11 | 4 | 16 | 6 |

**Supplementary Table 2**. Patient characteristics of the three cohorts. ADA = anti-drug antibody, ABT = antigen binding test, BE = bridging ELISA, IR = infusion reaction.

| Diagnosis | n | ADA+ (ABT) | ADA+ (BE) |
|---|---|---|---|
| Cohort 1A, IR+ (ADA+/ ADA-) | 19 | 12 | 12 |
| Spondyloarthritis | 9 | 5 | 5 |
| Peripheral arthritis | 7 | 4 | 4 |
| Other | 3 | 3 | 3 |
| Cohort 1B, IR- (ADA+ in BE) | 39 | 39 | 39 |
| Spondyloarthritis | 16 | 16 | 16 |
| Peripheral arthritis | 23 | 23 | 23 |
| Cohort 2, IR+ (ADA+/ ADA-) | 18 | 17 | |
| Rheumatoid arthritis | 18 | 17 | |
| Cohort 3, Infliximab treated | 83 | | 13 |
| Spondyloarthritis | 83 | | 13 |

**Supplementary Table 3.** Adverse events observed during or after infusion reaction (IR) determined for each IR+ patient from Cohort 1A and Cohort 2. Additionally, IgG anti-drug antibody (IgG-ADA) and IgE-ADA status is shown for each patient. Patients were considered positive if at least one sample had a detectable ADA level. IgG-ADA status was determined with an antigen binding test (ABT).

| | Observed adverse events | IgG-ADA+ | IgE-ADA + |
|---|---|---|---|
| Cohort 1A, IR+ (ADA+/ ADA-) | | | |
| 1 | Urticaria that subsided with medication, two days after infusion generalized urticaria | no | no |
| 2 | Urticaria, pruritus, dyspnea | yes | no |
| 3 | Despite premedication dizziness, malaise, chest tightness, nausea | yes | no |
| 4 | Despite premedication dizziness, diaphoresis, hypotension | yes | no |
| 5 | Erythema, pruritic superficial skin lesions | no | no |
| 6 | Despite premedication hypotension, diaphoresis | yes | no |
| 7 | Despite premedication perilabial edema | yes | no |
| 8 | Anxiety, improved after dexchlorpheniramine and hydrocortisone | yes | no |
| 9 | Despite premedication headache, malaise, abdominal pain, low back pain, improved after corticosteroid | yes | yes |
| 10 | Vomiting, itching, discomfort, improved after dexchlorpheniramine | yes | no |
| 11 | Pharyngeal edema, nausea, malaise, poor temperature regulation, improves after dexchlorpheniramine | no | no |
| 12 | Skin rash, conjunctival injection, dyspnea, palpitations, poor temperature regulation | no | no |
| 13 | Nausea, vomiting, back pain, sore throat, conjunctival injection, poor temperature regulation | yes | no |
| 14 | Despite premedication dyspnea, discomfort | no | no |
| 15 | Cutaneous rash, sweating, wheezing, improved with steroids and dexchlorpheniramine | no | no |
| 16 | Itching, thoracic rash | yes | no |
| 17 | Despite premedication generalized heat, dyspnea, edema of lips | yes | yes |
| 18 | Sore throat, generalized pruritus, improved after dexchlorpheniramine and corticosteroids | no | no |
| 19 | Chest tightness, dry cough, improved after dexchlorpheniramine and hydrocortisone | yes | no |

Table continues on page 129

**Supplementary Table 3.** *(continued)* Adverse events observed during or after infusion reaction (IR) determined for each IR+ patient from Cohort 1A and Cohort 2. Additionally, IgG anti-drug antibody (IgG-ADA) and IgE-ADA status is shown for each patient. Patients were considered positive if at least one sample had a detectable ADA level. IgG-ADA status was determined with an antigen binding test (ABT).

| | Observed adverse events | IgG-ADA+ | IgE-ADA + |
|---|---|---|---|
| Cohort 2, IR+ (ADA+/ ADA-) | | | |
| 1 | Edema, erythema, wheezing, tachycardia | yes | no |
| 2 | After 30 minutes unwell, hot flushes, dyspnea | yes | no |
| 3 | After 30 minutes erythema, chest tightness | yes | no |
| 4 | Chest tightness, dyspnea | yes | no |
| 5 | Pale, dizziness, urticaria | yes | no |
| 6 | Hot flushes, nausea | yes | no |
| 7 | Mild reaction, erythema, dyspnea, urticaria, pruritus | yes | no |
| 8 | Dyspnea, tachycardia | yes | no |
| 9 | Headache, hot flushes | no | no |
| 10 | After 20 minutes facial edema, hypotension, prolonged expiration | yes | no |
| 11 | Dyspepsia, dyspnea, palpitations | yes | no |
| 12 | Periorbital edema | yes | no |
| 13 | Hot flushes, edema, urticaria | yes | yes |
| 14 | Dyspnea, hot flushes | yes | no |
| 15 | Headache, nausea, itching | yes | no |
| 16 | Hot flushes, dyspepsia, mucosal edema | yes | no |
| 17 | Fever, unwell | yes | no |
| 18 | Fever, hot flushes | yes | yes |

# SUPPLEMENTARY METHODS

**Patients**

For the development of human monoclonal anti-infliximab antibodies, heparin blood was collected from two patients with high IgG-ADA (anti-drug antibodies) titers and further treated as described below.

A total of 626 sera of 213 patients were tested on presence of IgE-ADA. To determine the general IgE-ADA positivity in IgG-ADA positive patients, sera positive for IgG-ADA (n=54) were selected from our diagnostic department. These samples were sent to Sanquin Diagnostic Services for evaluation of infliximab and IgG-ADA concentration using an antigen binding test (ABT). For these samples no ethical approval was obtained, since materials used for this study were leftovers from samples taken for routine diagnostic purposes. Materials were used anonymously without any connection to clinical data.

To determine the association of IgE-ADA with infusion reactions (IR), Cohort 1A and Cohort 2 were used. *Cohort 1A* consisted of 19 Spanish patients that were selected based on presence of IR, irrespective of ADA status. *Cohort 2* consisted of 18 Dutch patients, selected based on presence of IR, irrespective of IgG-ADA status. *Cohort 1B* contained patients from the same centre as Cohort 1A, however these patients did not have an IR, but were selected on ADA positivity in the bridging ELISA.

To determine the incidence of IgE-ADA in infliximab treated patients, Cohort 3 was used. *Cohort 3* is a longitudinal retrospective cohort (n=83) that contained infliximab treated patients from Spain of which 13 patients were ADA+ in the bridging ELISA.

Patient characteristics are shown in Supplementary Table 2 and for IR+ patients adverse events are described in Supplementary Table 3. Generally, infusion reactions described in this study were mild or moderate, and all patients recovered without sequelae. All patients gave informed consent and the study was approved by the local medical ethical committees in Spain and The Netherlands.

Sera were evaluated for the presence of ADA using an antigen-binding test (ABT, Sanquin, The Netherlands; or a bridging ELISA (BE). The bridging ELISA is less drug-tolerant than the ABT, and therefore only identifies antibodies with the highest impact on infliximab concentrations and clinical efficacy.[2] For Cohort 1B, with selection based on BE+, all patients were also found positive in the ABT.

### Isolation, proliferation and identification of infliximab specific single B-cells

From blood of two IgG-ADA+ patients, infliximab specific B-cells were isolated essentially as described previously, with the modification that CD19-PerCP-Cy5.5 positive (BD Biosciences) and IgD-PE negative (BD Biosciences) cells were isolated, and infliximab specific cells were further sorted using either biotinylated infliximab (Remicade, Janssen Biotech, Inc.) subsequently labelled with streptavidin-APC (BD Biosciences) or infliximab $F(ab)_2$ directly labeled with AF650 (Thermo Fisher, labeled according to the manufacturer's instructions).[3] Isolated B-cells were plated 1 cell per well in 96-well flatbottom plates, in the presence of $1*10^4$ irradiated (30 Gy) murine 3T3 fibroblast cells transfected with CD40L, and cultured as described before.[3] After 9 days, B cell supernatants were tested for anti-infliximab antibody production using a bridging ELISA analogously as described for adalimumab.[3]

### Determining the subclass of monoclonal anti-infliximab antibodies

To determine the IgG subclass, a 96-well flatbottom MaxiSorp plate was coated with 1 µg/ml anti-human-IgG1, 10 µg/ml anti-human-IgG2 or anti-human-IgG3, or 5 µg/ml anti-human-IgG4 (MH161-1 (Sanquin), HP6014 (Abcam), MH163-1 (Sanquin), MH164-4 (Sanquin), respectively) in PBS and incubated overnight. Plates were washed five times with PBS-T after which supernatants (1:6.6 diluted in High Performance ELISA buffer (HPE, Sanquin)) were added and incubated for 1 hour. After washing, samples were incubated with 1 µg/ml anti-IgG-HRP MH16.1 (Sanquin) in HPE and incubated for an hour. Plates were washed and additionally developed using substrate solution as described before.[3]

### Production of recombinant human antibodies

From wells which were found positive for anti-infliximab antibodies, RNA was isolated using Trizol (Peqlab). cDNA synthesis, NESTED PCR and RACE PCR were performed using the Clontech SMART cDNA synthesis kit using immunoglobulin specific primers.[4] The light chain of the monoclonal antibodies was determined based on presence of a PCR product using kappa and lambda specific primers. RACE PCR products for VL and VH were sequenced and the nucleotide sequence analysis was performed using IMGT/V-QUEST.[1] Synthetic DNA constructs for VL, VH and the constant domain of kappa light chain, IgG1 (IGHG1*03), and IgE (IGHE*02) were ordered (Life Technologies) and cloned into pcDNA3.1 (Invitrogen) expression vectors as described before.[5] These expression vectors were used for transient transfection of HEK293F cells with 293fectin and OptiMEM (Invitrogen), using the Freestyle HEK293F expression system (Invitrogen) according to the manufacturer's instructions.

### Western blot analysis

IgE anti-infliximab (50 or 500 IU/lane) was size-separated using a NuPage 4-12% Bis-Tris gel (Invitrogen). Precision plus protein All Blue (Bio-RAD) was used as standard. Proteins were transferred onto a nitrocellulose membrane (Life Technologies) using an iBlot apparatus (Invitrogen). The membrane was blocked for 2 hours in PBS containing 0.1% Tween-20 and 1% western blocking reagent (WBR, Roche), followed by overnight incubation at 4°C with either biotinylated anti-IgE or biotinylated infliximab diluted in PBS containing 0.1% Tween-20 and 0.5% WBR. Membranes were washed three times for 15 minutes with washing buffer (PBS containing 0.1% Tween-20), followed by 30 minutes incubation at room temperature with streptavidin-HRP (1:1000, GE Healthcare) diluted in PBS containing 0.1% Tween-20 and 0.5% WBR. After washing three times with washing buffer, and two times washing with PBS, membranes were developed using Pierce™ Western blotting substrate (Thermo Scientific) according to the manufacturer's instructions. The membranes were analysed using the ChemiDoc™ MP (BioRad) with Image Lab 5.0 software.

### Generation of F(ab')$_2$ fragments

F(ab')$_2$ fragments were generated using pepsin, essentially as described previously.[6]

### IgE-ADA ELISA

To measure IgE-ADA antibodies maxisorp ELISA plates were coated overnight at 4°C with 2 µg/ml omalizumab (monoclonal anti-IgE, Xolair, Novartis) F(ab')$_2$ in PBS. After five times washing with PBS-T, plates were incubated for 1 h at room temperature with 100 µl of samples 10x diluted in HPE+ (HPE (Sanquin) supplemented with 1 mg/ml IVIg (Nanogam, Sanquin) and 50 µg/ml IVIg F(ab')$_2$, both as blocking reagent), additionally supplemented with 50 µg/ml reduced and alkylated natalizumab (Tysabri, Biogen, reduced and alkylated by incubating with DDT and iodoacetamide, respectively), also as blocking reagent. Plates were washed five times with PBS-T. Then 100 µl of 1 µg/ml biotinylated infliximab F(ab')$_2$ in HPE+ was added followed by overnight incubation at 37°C. Plates were washed five times with PBS-T, and incubated with streptavidin-poly-HRP diluted 1:40 000 in HPE for 1 hour at room temperature. Plates were washed five times with PBS-T, and TMB ELISA substrate solution (Interchim) twice diluted in $H_2O$ was added to each well. After 5 minutes the reaction was stopped by the addition of 2 M $H_2SO_4$. Absorbance was measured at 450 nm and 540nm. The recombinant IgE anti-infliximab was used as calibrator. The concentration of the calibrator was determined by measurement of total IgE with an in-house IgE ELISA, and samples were compared to a serum pool with an amount of IgE that was previously calibrated to the WHO standard 75/502. A lower limit of detection was determined as the mean + 2.6 SD of 50 infliximab-naive spondyloarthritis patients resulting in a technical cut-off of

0.08 IU/ml. Values above 0.1 IU/ml were determined positive, and a cut-off of 0.35 IU/ml as commonly used in clinical practice was used for clinical relevance.

**Serum IgE depletion**

Anti-IgE (clone 16B7, Sanquin) was coupled to CNBr-activated Sepharose (GE Healthcare) according to the instructions of the manufacturer. An aliquot (0.2 mL) of serum was applied to a column of anti-IgE Sepharose and flow through was collected, followed by washing of the column with PBS and elution with glycine pH 2.5. The pH of the eluate was neutralized with 2M Tris pH 9.0.

# REFERENCES


1. Brochet X, Lefranc M-P, Giudicelli V. IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. *Nucleic Acids Res.* 2008; 36(Web Server issue):W503–8.
2. Hart MH, de Vrieze H, Wouters D, et al. Differential effect of drug interference in immunogenicity assays. *J. Immunol. Methods*. 2011;372(1-2):196–203.
3. van Schouwenburg PA, van de Stadt LA, de Jong RN, et al. Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralisation. *Ann. Rheum. Dis.* 2013;72(1):104–9.
4. Dohmen SE, Verhagen OJHM, Muit J, Ligthart PC, van der Schoot CE. The restricted use of IGHV3 superspecies genes in anti-Rh is not limited to hyperimmunized anti-D donors. *Transfusion*. 2006;46(12):2162–2168.
5. van Schouwenburg PA, Kruithof S, Votsmeier C, et al. Functional analysis of the anti-adalimumab response using patient-derived monoclonal antibodies. *J. Biol. Chem.* 2014;289(50):34482–34488.
6. van Schouwenburg PA, Bartelds GM, Hart MH, et al. A novel method for the detection of antibodies to adalimumab in the presence of drug reveals “hidden” immunogenicity in rheumatoid arthritis patients. *J. Immunol. Methods*. 2010;362(1-2):82–88.