

Drug-Specific Th2 Cells and IgE Antibodies in a Patient with Anaphylaxis to Rituximab

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Established Facts

- Biological agents are immunogenic and anti-drug antibodies may sustain an adverse infusion reaction.
- Rituximab frequently causes immediate infusion reactions, usually classified as cytokine release syndrome.

Novel Insights

- Rituximab can induce adverse reactions through an IgE-mediated mechanism.
- The presence of drug-specific Th2 cells and serum IgE antibodies in a patient with severe reactions to rituximab is reported.

Key Words

Anaphylaxis • Anti-rituximab antibodies • Biological agents
• Drug allergy • Drug-specific T cells

Abstract

Rituximab (RTX) is currently used in the treatment of lymphoproliferative diseases and of several rheumatologic disorders and is a frequent cause of acute infusion reactions, usually classified as cytokine release syndrome (CRS). Some infusion reactions to RTX raise concern for immediate type I hypersensitivity, even if to date RTX-specific IgE antibodies have not been reported. To improve knowledge of the mechanisms of

reactions to RTX, we investigated humoral and cellular immune responses to this drug in a patient suffering from rheumatoid arthritis who displayed two immediate infusion-related reactions. RTX-exposed tolerant patients and healthy untreated subjects were used as controls. Non-isotype-specific and IgE anti-RTX antibodies were positive in the serum samples collected from the reactive patient but not in those from the control groups. Only the reactive patient also displayed skin testing positivity with RTX. More importantly, RTX-stimulated peripheral blood mononuclear cells from the reactive

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patient, but not from the controls, displayed a dose-dependent proliferative response associated with a Th2 cytokine production profile. Our results show the presence of RTX-specific Th2-type cells and IgE antibodies, thus suggesting that type I hypersensitivity may be an additional mechanism to CRS in the development of RTX reactions.

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Introduction

Rituximab (RTX) is a chimeric IgG1 monoclonal antibody (mAb) directed to the CD20 molecule expressed by B cells, currently used in the treatment of lymphoproliferative diseases and several rheumatologic disorders. Notably, RTX is one of the most frequent causes of acute infusion reactions. It is currently agreed that RTX reactions are due to a massive cytokine release, leading to a potentially severe condition defined as cytokine release syndrome (CRS) and usually occur during the first infusion [1]. However, some infusion reactions to RTX raise concerns for immediate type I hypersensitivity [2]. We know that clinical manifestations of IgE-mediated reactions and CRS partially overlap; indeed, rash, hypotension, nausea, tachycardia and shortness of breath have been described in both types of reaction. Fever, although not classically considered to be a feature of immediate hypersensitivity, might be correlated to pyrogens released by mast cells during an IgE-mediated reaction [1–4].

Recent studies have reported the presence of serum anti-RTX antibodies (Abs) in some treated patients [5]. Although anti-drug antibodies (ADAs) are mostly represented by the IgG isotype [6], a proportion of hypersensitivity reactions have been related to the presence of IgE ADAs for several biological agents [7]. ADAs represent the result of the adaptive immune response to the drug, which also implies the presence of drug-specific memory T cells. To date there have been no reports on RTX-specific T cells and IgE Abs.

This study aimed firstly to show the association of ADAs and, particularly of IgE Abs, with RTX-induced reactions, and secondly to investigate the presence of RTX-specific T cells displaying a Th2 cytokine profile.

Case Report

In April 2009, a 46-year-old woman suffering from rheumatoid arthritis was submitted to a first RTX cycle ($2 \times 1,000$ mg) which was well tolerated. The patient had no known prior exposure to any other biological agents. Ten months later a second

course of RTX treatment was started. Just before the end of the second infusion she complained of itching, nausea, abdominal pain, mild respiratory distress and throat constriction (fig. 1a). She was treated with oral hydroxyzine (25 mg) and i.v. 6-methylprednisolone (80 mg), resulting in a rapid clinical improvement. In December 2010, the patient's arthritis worsened and she was given a third course of RTX treatment. A few minutes after the beginning of the third infusion (fig. 1a), she developed a severe reaction, suffering bronchospasm, hypotension (80/40), urticaria, abdominal pain and dizziness, which needed emergency treatment. Premedication with acetaminophen (500 mg), hydroxyzine (25 mg) and methylprednisolone (100 mg) had been performed before each RTX infusion. During the RTX cycles, performed at the Hematology Unit, several serum samples had been collected and stored, but not immediately after the two reactions (fig. 1a). In the meantime circulating B cells had been monitored (fig. 1b). In January 2011, she was admitted to our unit for an immunological evaluation. The patient referred seasonal rhinoconjunctivitis but not urticaria/angioedema or drug intolerance. Upon admission skin tests had been positive for grass pollen and negative for foods, including beef and pork. High serum levels of rheumatoid factor (694 U/ml) and anticitrullinated peptide Abs (89.5 U/ml) were present. Circulating B cells were undetectable and a follow-up was performed (fig. 1b).

Anti-RTX Abs were measured by a homemade double-bridging ELISA assay, by using horseradish peroxidase-labeled RTX as a detecting reagent, following the recommendations for validation of the assay procedure as previously reported [8, 9]. ADAs were detectable in serum samples collected after the first (at low level) and the second drug reaction, while no preformed ADAs were found (fig. 2a).

The monitoring of anti-RTX Abs titer showed a progressive decrease with a negative result 12 weeks afterwards (fig. 2a). RTX-specific IgE assessed by ImmunoCAP assay were present in sera collected after the two infusion reactions (samples 4 and 6), the first displaying lower levels than the second one (fig. 2b). Serum samples from 18 RTX-exposed tolerant patients with rheumatoid arthritis and 10 healthy subjects were used as controls. RTX-treated patients were exposed to 2 cycles of RTX 2–4 weeks before blood sampling: $2 \times 1,000$ mg/cycle, mean value ($\pm$ SE) of time between the two cycles 10.2 ± 2.6 months. Nonisotype-specific and IgE ADAs were not detected in 18 RTX-tolerant patients and 10 untreated control subjects (data not shown). A dose-dependent inhibition of non-isotype-specific and IgE ADA levels was observed when the patient's serum was preincubated with increasing doses of RTX and murine IgG, but not of another mAb (cetuximab; fig. 2c and data not shown). Skin testing with RTX was performed 3 weeks after the second reaction, as previously described [2, 10]. Prick testing with RTX (10 mg/ml) was negative, while intradermal testing at immediate reading with 1:100 drug dilution, but not with the control (diluent), resulted positive (wheal 12×12 mm, flare 18×20 mm).

The development of ADAs suggest a complete adaptive secondary response to the drug involving T and B cells. We then investigated the presence of RTX-specific T cells, using a two-step procedure of stimulation. Peripheral blood mononuclear cells (PBMCs) were initially cultured in medium plus 5% heat-treated human serum, in the absence or presence of RTX for 7 days and then restimulated with the drug for an additional 5 days. A dose-dependent RTX-specific proliferative response was observed,

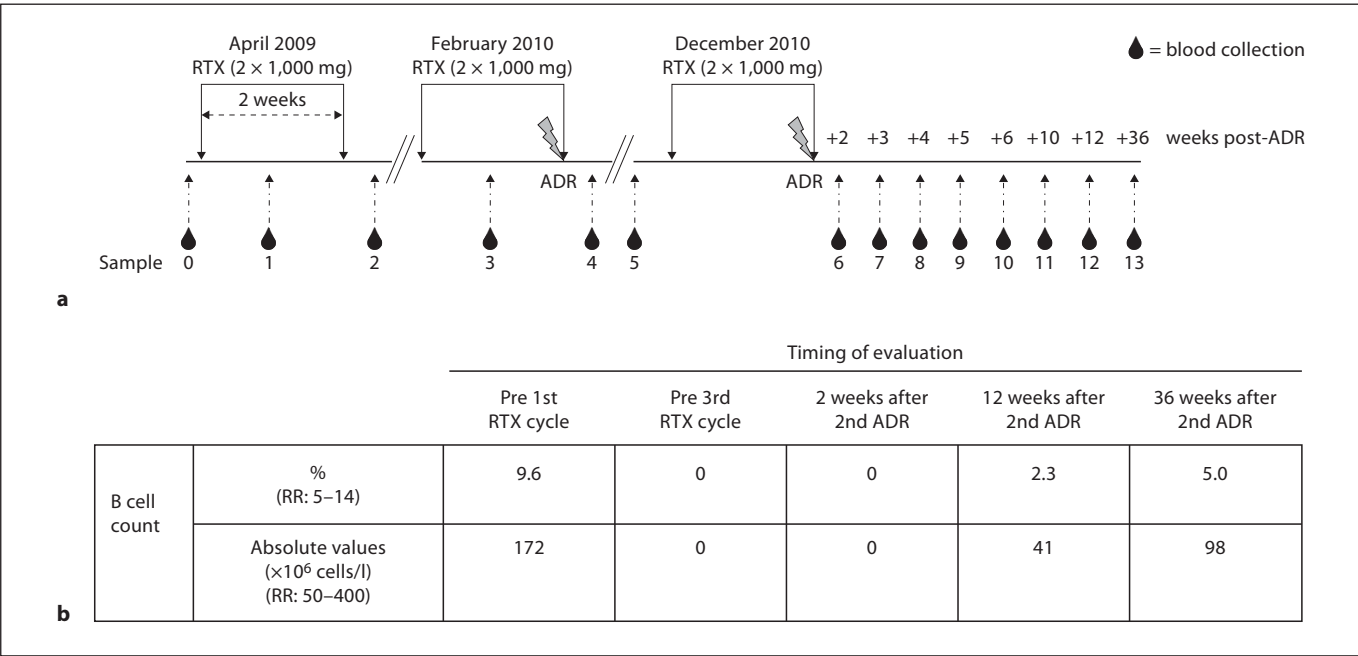


Fig. 1. a Timing of RTX therapy, adverse reactions and serum sampling. Sample 0: before the 1st cycle of RTX; samples 1 and 3: 1 week after RTX infusion; samples 2, 4 and 6: 2 weeks after each RTX infusion; sample 5: 2 weeks before the 3rd RTX cycle; samples 7–13, collected 3, 4, 5, 6, 10, 12 and 36 weeks after the second RTX reaction. **b** Kinetics of B cell count during RTX cycles. ADR = Adverse drug reaction; RR = reference range.

peaking in the seventh week and becoming undetectable in the twelfth week. No drug-specific proliferation was obtained with PBMCs from either the RTX-exposed disease-matched patients ($n = 8$) or the healthy donors ($n = 10$; fig. 3a). More importantly, high levels of IL-13, but not IFN- γ , were detected in 5-day culture supernatants of PBMCs from the reactive patient stimulated in vitro with RTX in the culture conditions mentioned above (7 and 5 days; fig. 3b). Cytokines were not detectable in control culture supernatants (data not shown). The proliferative response was inhibited in a dose-dependent manner by the anti-class II major histocompatibility complex (MHC), but not by the isotype control mAbs (data not shown). Of note, in the same experimental conditions, we observed a concomitant inhibition of RTX-induced IL-13 production (fig. 3b).

Discussion

The clinical features, including urticaria, bronchospasm, abdominal pain and dizziness, associated with the positivity of both in vitro and in vivo tests, suggest that an IgE-mediated mechanism may be responsible for the patient's reactions. To our knowledge, serum IgE Abs specific for RTX have not been reported, even though a type I hypersensitivity mechanism could al-

ready have been hypothesized on the basis of positive skin testing [2].

Clinical manifestations of type I hypersensitivity reactions and CRS may overlap, as is well known and previously reported [4]. It is also likely that CRS and type I hypersensitivity response cannot be mutually exclusive, since Fc ϵ RI-driven mast cell/basophil activation can lead to the release of mediators involved in CRS [11].

The two reactions experienced by the patient did not occur at the first cycle of therapy and/or at the first infusion, thus suggesting an immunization phase and an adaptive secondary response to the drug involving T and B cells. Furthermore, in contrast to the results obtained with cetuximab [12], we excluded preexisting sensitization in our patient.

Besides RTX-specific IgE, we also showed a RTX-specific Th2 response that may be able to sustain the production of drug-specific IgE. The inhibition of proliferation by the anti-class II MHC mAb strongly suggests that the majority of the proliferative response is due to antigen-specific CD4 $^{+}$ T cells. Furthermore, the concomitant down-regulation of RTX-induced IL-13 production implies that the release of IL-13 is sustained by drug-specif-

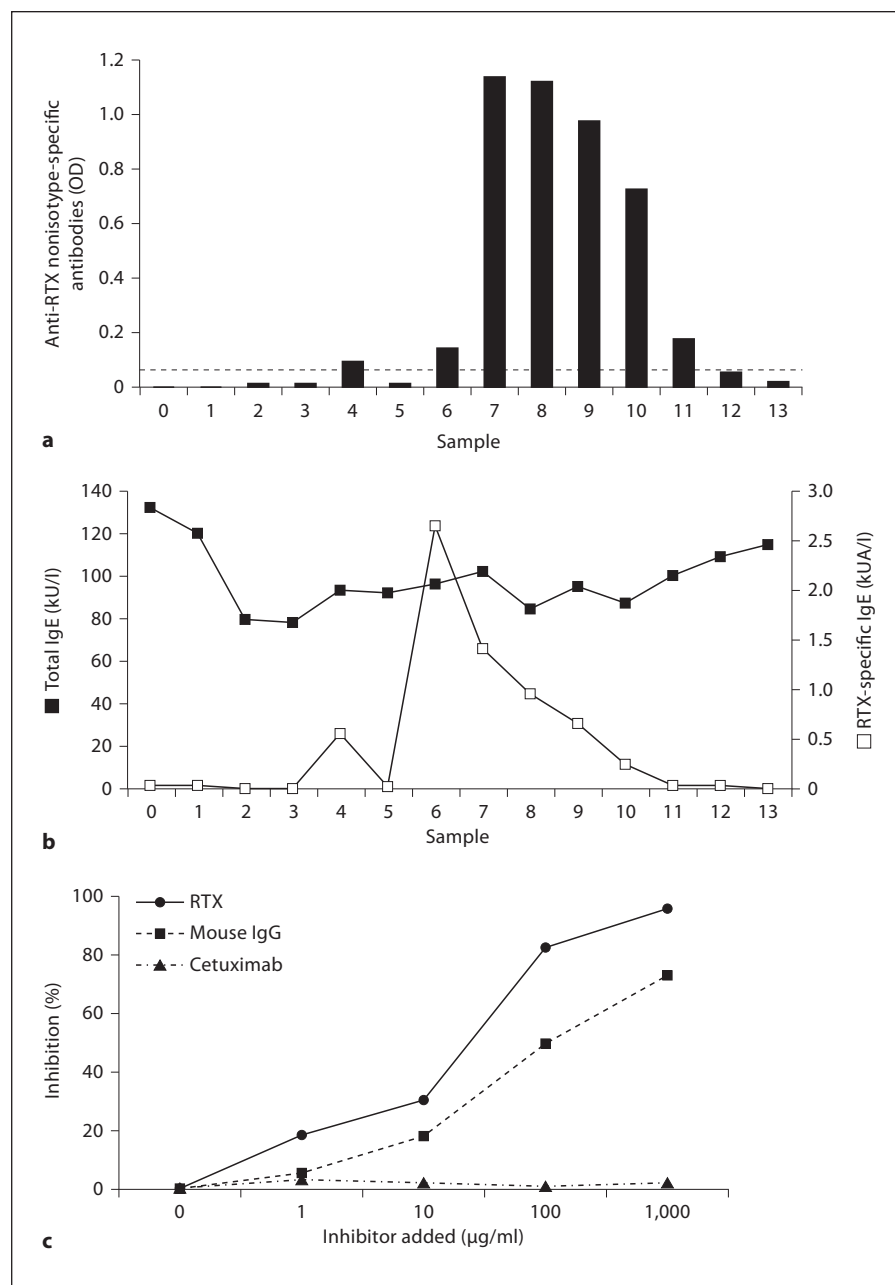
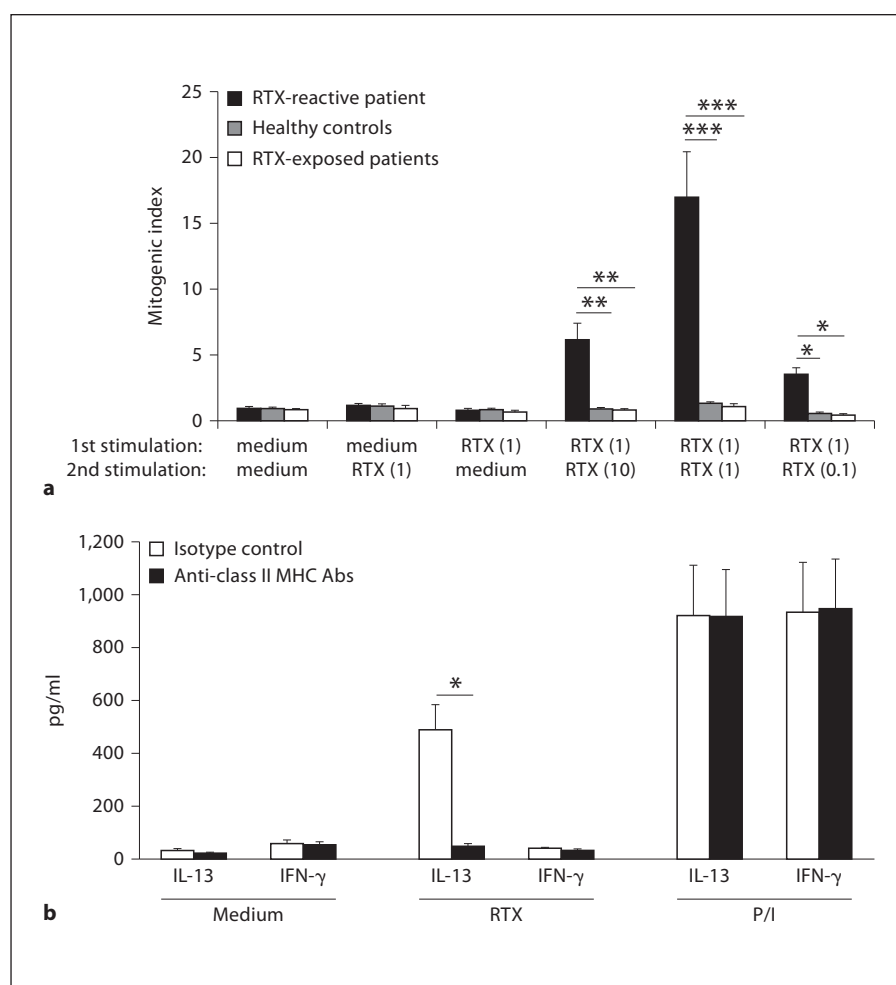


Fig. 2. **a** Anti-RTX non-isotype-specific antibodies in the reactive patient. Results are expressed as mean values of optical density (OD) of each serum sample (dilution 1:100) analyzed in triplicate. The dotted line indicates the cut-off point (0.065) calculated as the 95th percentile of results obtained in pre-study validation from 50 sera of RTX-unexposed healthy subjects. **b** Total and RTX-specific IgE antibodies in sera of the reactive patient. Cut-off value for RTX-specific IgE: 0.10 kUA/l. **c** Inhibition of RTX-specific IgE (sample 6) with increasing doses of RTX, cetuximab and murine polyclonal IgG.

ic T (Th2) cells. The RTX-specific memory T cells were not detectable immediately after the second reaction, probably due to their very low frequency and/or in vivo exhaustion. The kinetics of T cell response also suggests the appearance of a pool of memory T cells following the antigen administration, which thereafter slowly decreases (homing to lymph nodes?). The progressive decrease of ADA titer and T cell response a few weeks after the reaction indicates how the timing of in vitro tests is a key issue

for detecting the adaptive response to a biological agent. The break of tolerance and the induction of immunogenicity to biological products is a complex and quite variable phenomenon, essentially involving the recognition of different unknown epitopes and homeostatic mechanisms. It is hard to compare the immunogenicity of biological agents with that of other drugs, such as β -lactams, for which a T cell proliferative response can be observed many years after the reaction, without any further expo-

Fig. 3. a Proliferative response to RTX of PBMCs from the reactive patient, RTX-tolerant patients suffering from rheumatoid arthritis (n = 8) and healthy controls (n = 10). PBMCs (10^6 cells/ml) were cultured in complete medium plus 5% human serum in the absence or presence of RTX (1 μ g/ml) for 7 days. Cells (2×10^5) were then restimulated in micro wells in the same medium with different doses of RTX (0.1–10 μ g/ml) for 5 days and then culture supernatants were collected. The cultures were counted after 16 h of pulsing with 0.5 μ Ci 3HTdR/well (Amersham, UK). PBMCs from the reactive patient were analyzed five subsequent times from week 3–7 after the 2nd reaction; PBMCs from RTX-exposed tolerant patients were analyzed 2–4 weeks after the last RTX infusion. Results are expressed as mean values $\pm$ SEM of the mitogenic index, calculated as the ratio between the mean value of counts per min obtained in cultures with RTX and those obtained in medium alone. A value ≥ 3 was regarded as positive. **b** Cytokine levels were measured in 5-day culture supernatants collected as in **a**; cells were cultured in the presence of anti-class II MHC or isotype control mAbs (10 μ g/ml, Becton Dickinson, San Jose, Calif., USA). IL-13 and IFN- γ were measured by commercial ELISA kits (R&D Systems, Minneapolis, Minn., USA). P/I: PMA (50 ng/ml) plus ionomycin (500 ng/ml); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



sure to the drug [13]. Of note, the persistence of T cell response to β -lactams has been ascribed to an ongoing stimulation by small and hidden amounts of antibiotics in foods [14].

A challenging point concerns the demonstration of a humoral response to RTX despite B cell depletion induced by the treatment. Indeed, B cells are only transiently depleted upon RTX treatment, but they return to the previous normal range after a variable period of time. In our patient B cell absolute numbers were restored within 9 months [15].

Several reports show that RTX treatment does not lead to a complete disappearance of autoantibodies in patients suffering from autoimmune diseases [15, 16]. Interestingly, the serum levels of Abs to microbial antigens did not change significantly upon RTX therapy [16, 17]. Otherwise, the development of ADAs during RTX treatment is a well-described phenomenon. It has been observed that

total IgM and IgE production is due to short-lived plasma cells and that their titer decrease, even though they are not totally abolished after RTX treatment [18]. In our case, as expected, we observed a decrease of total IgE serum levels of about 40% (39.8%, from 132 to 79.2 kU/l; fig. 2b). More importantly, the reduction of total IgE levels did not influence the up-growth of RTX-specific IgE after the two reactions.

Our data also provide evidence that T cell priming to RXT can be induced as the treatment does not compromise naive T cells and dendritic cell functions, as reported [19]. The results also suggest that RTX-specific T cells may cooperate with CD20 B cell precursors to elicit a valid humoral response.

Another relevant issue of this study concerns ADA specificity: the high degree of inhibition of RTX-specific IgE (as that of nonisotype-specific ADAs) by polyclonal murine IgG strongly suggests that humoral response to

the drug is mainly directed to the murine frame shift of therapeutic mAb. In fact, it is well known that xenoantigens (murine sequences) display a stronger immunogenicity than the idiotypic or allotypic determinants of the human part of mAb.

In conclusion, to our knowledge, this study is the first demonstration of the presence of both cellular (Th2 cells) and humoral (IgE) response to RTX and suggests that type I hypersensitivity may be an additional mechanism to CRS in the development of infusion reactions to the drug.

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