Rituximab for the Treatment of Graves' Orbitopathy

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Abstract

The contribution of B cells to human autoimmune disease has recently been underscored because of the therapeutic benefit of B-cell depleting therapies. B cells are involved in the production of autoantibodies and in CD4+ T-cell activation and control of T-cell function and inflammation, through cytokine production. B cells are also important antigen presenting cells. Rituximab (RTX) has been used off-label in various autoimmune disorders and has been shown to effectively deplete mature and memory CD20+ B cells, but not long-lived plasma cells. The rationale of RTX use in Graves' disease (GD) and Graves' orbitopathy (GO) relies on its putative effect on pathogenic autoantibodies causing hyperthyroidism. RTX in patients with active GO has been shown to significantly affect the inflammatory activity and severity of GO. Caution is suggested before proposing RTX as a novel therapeutic tool in this disease until randomised controlled studies are available. Should preliminary observations be confirmed, an optimal strategy for controlling the progression of GO would be to pursue B-cell depletion shortly after diagnosis and not as an additional therapeutic option when standard immunosuppression has failed.

Keywords

Graves' orbitopathy, Graves' disease, B lymphocytes, CD20, rituximab, thyroid stimulating hormone receptor (TSHR) antibodies, thyroid peroxidase (TPO) antibodies, Tg antibodies, CD68, CD163

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Functions of B Cells and Their Role in Autoimmune Disease

Although B lymphocytes are known to contribute to the pathogenesis of autoimmune disease through autoantibody production,[1] many recent studies of B-cell function carried out in experimental animal models have shown that they possess other functions such as CD4+ T-cell activation[2] and control of T-cell function and inflammation through cytokine production. B cells in fact are involved in multiple pathways of the immune system in autoimmune disease by combining their multipurpose cellular and humoral functions (see *Figure 1*).[3] B-cell contributions to human autoimmune disease have recently been reconsidered due to the therapeutic benefit of B-cell depleting therapies.[4]

Antibody Production

Antigens generally activate mature B cells to generate antibody secreting plasmablasts, and short-lived plasma cells that secrete antigen-specific antibodies.[5] Following antigen-specific proliferation, B cells enter into the germinal center microenvironment, where they diversify their antigen receptors and generate pools of long-lived memory B cells[6] that are responsible for producing and maintaining serum antibody levels.[7] In autoimmune diseases, autoantibodies may be pathogenic through direct binding to specific receptors (e.g. the thyroid stimulating hormone receptor [TSHR] on the thyrocyte membrane in Graves' disease[GD]) or through the formation of immune-complexes in tissues that locally activate complement reactions and induce inflammation.

Antigen Presentation and Co-stimulation

B cells are important antigen-presenting cells in the initiation of immune responses.[8,9] Autoantigen stimulation in B-cell-depleted mice results in reduced antigen-specific CD4+ T-cell activation, which underscores that B cells contribute to the initiation of autoimmune reaction.[10]

Effects on Lymphoid Tissue Neogenesis and Production of Cytokines

B cells are also involved in lymphoid tissue formation[11] and in mice, after B-cell depletion, it is not possible to identify lymphoid follicles within the spleens.[12] Furthermore, B cells produce numerous cytokines including interleukin (IL)-10, IL-4, IL-6, lymphotoxin-α, transforming growth factor-beta (TGF-β and interferon-gamma [IFN-γ]).[13]

Regulatory B Cells

Regulatory B cells (B regs) or B10 cells are an IL-10-producing subset of B-cells.[14] Studies in mice indicate that IL-10-producing B cells play important roles in the suppression of autoimmune and inflammatory disease. IL-10 produced by B cells has been shown to downregulate autoreactive immune mechanisms in collagen-induced arthritis[14] and inflammatory bowel disease.[15] As a consequence, B-cell elimination using rituximab (RTX) was in fact associated with exacerbation of ulcerative colitis[16,17] and psoriasis,[18] both conditions representing Th1-mediated autoimmune conditions.

Table 1: Clinical Characteristics of Patients with Graves' Orbitopathy Treated with Rituximab

Study	Number of Patients Treated	RTX Dose	CAS Before Therapy	CAS After Therapy (16 Weeks)	Severity After RTX	Number of Patients with Side Effects	GO Relapse
Salvi et al., 2006–2007[32,34]	9	1 g twice with 2-week interval	4.7	1.8	All improved	3 (minor)	No
El Fassi et al., 2007[42]	2	375 mg/m^2 weekly for 4 weeks	5.5	1.5	All improved	1 (minor)	No
Khanna et al., 2010[35]	6	1 g twice with 2-week interval	5.5	1.3	All improved	2 (minor) 1 (major, cardiac death, likely unrelated to therapy)	No
Silkiss et al., 2010[36]	12	1 g twice with 2-week interval	5.5	1.9	All improved	None	No
Mitchell et al., 2010[38]	6	500 mg or 1 g at 2-week interval	5.5	2	5 patients 1 patient unchanged	None	Not reported
Krassas et al., 2010[40]	1	1 g twice with 2-week interval	7	7	Worsened	Not reported	Yes
Madaschi et al., 2010[39]	1	1 g twice with 2-week interval	5	0	Improved	No	No
Salvi et al., 2011[41]	3	100 mg single dose	5.3	1.6	All improved	2 (major but transient)	No

CAS = clinical activity scores; GO = Graves' orbitopathy; RTX = rituximab.

B-cell Depletion with Rituximab

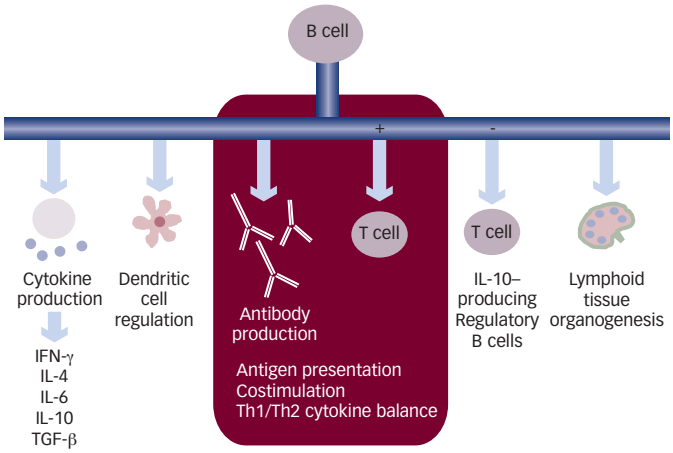
RTX, a chimeric mouse-human monoclonal antibody targeting the CD20 antigen, has been used off label in various autoimmune disorders but is approved for clinical use only in non-Hodgkin's lymphoma and for the treatment of moderate-to-severe rheumatoid arthritis (RA) in patients who did not respond to TNF inhibitors. RTX depletes more than 95 % of mature B cells in blood and primary lymphoid organs after two days by a single RTX treatment in mice.

CD20 is a human B lymphocyte-specific antigen expressed on immature to mature B cells, as well as memory B cells but not on stem cells and B cell precursors. Most importantly, CD20 is not expressed on antibody-producing plasma cells (see *Figure 2*).[19] As a consequence, therapeutic targeting of CD20+ cells removes B lymphocytes in all intermediate stages of B cell maturation and, in addition, activated memory B and short-lived plasma cells by depleting their immediate precursors. Peripheral short-lived plasma cells return almost to baseline levels six to 10 months after treatment, at the time of B cell repopulation. As therapy does not affect B cell precursors or long-lived plasma cells residing in the bone marrow,[20,21] antibody production is maintained over a long period of time, even without the contribution from memory cells.[7,22] This is why immunoglobulin levels may not change throughout the period of peripheral B-cell depletion,[23,24] even after multiple courses of treatment. In humans, it is yet to be understood whether RTX is effective for the treatment of autoimmune diseases because of its direct B-cell depleting action or by indirectly affecting autoantibody production. Consequently, novel biomarkers of its mechanism of action have to be sought. In particular, studies should address why response does not always correlate with complete B-cell depletion, as has been reported in some patients with RA.[24,25]

Pharmacokinetics

The majority of studies on RTX pharmacokinetics and pharmacodynamics have been performed in patients with B-cell lymphomas and have shown that serum concentration of RTX directly correlated with response and inversely correlated with tumour mass.[26] Variable half-lives (11–105 hours) may thus result from the different tumour burden and also from the changes of CD20 expression in malignant B cells consequent to repeated RTX administration.[27] In RA, RTX half-life has been reported to be much longer, up to 20 days after two doses of 1,000 mg, the dose being used in most autoimmune diseases,[28] and the reason for this difference is not known. Dosing studies are also ultimately important in view of RTX dose costs: should lower doses in autoimmune disease be as effective as the higher doses, employed so far based on studies carried out in lymphomas, their costs would more likely be affordable by specialised clinical centres.[27]

Figure 1: The Multiple Functions of B Lymphocytes

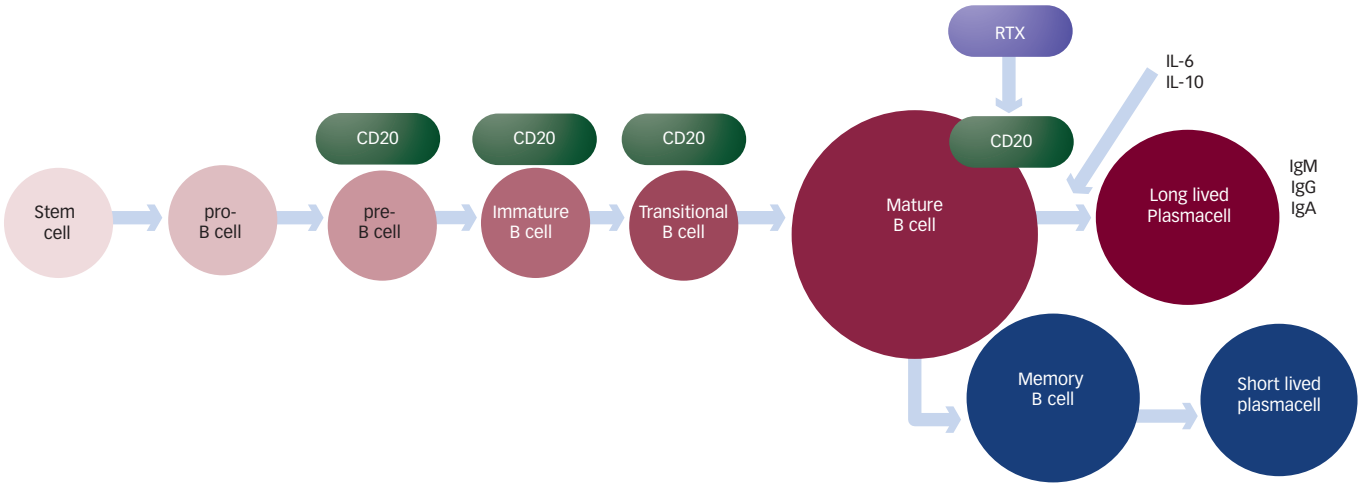


IFN-γ = interferon-gamma; IL = interleukin; TGF-β = transforming growth factor-beta.

Safety Profile

Infusion related reactions are the most frequently reported side effects of RTX.[29] Release of pro-inflammatory cytokines from macrophages, monocytes, lymphocytes and NK cells is the underlying mechanism. Additional mechanisms such as activation of complement

Figure 2: Expression of the CD20 Antigen Throughout the Maturation Steps of B Lymphocytes as a Potential Therapeutic Target for Direct B-cell Depletion



Ig = immunoglobin; IL = interleukin; RTX = rituximab.

Table 2: Comparison of Rituximab versus Glucocorticoid Therapy for Graves' Orbitopathy in Patients with Active Disease

		RTX	Glucocorticoids
Number of patients		9	20
Time to reach CAS<3		4 weeks	6 weeks
Response rate (%)	Activity	100	80
	Severity	88	75
Side effects (%)		33	45

CAS = clinical activity scores; RTX = rituximab.
Source: Salvi et al., 2007.[34]

cascade may be responsible of acute reactions caused by RTX. These reactions may be present in about 10 % of patients at first infusion, they can be severe but reversible. Among major side effects, progressive multifocal leukoencephalopathy (PML) has rarely been reported in patients receiving RTX, especially those with systemic lupus erythematosus (SLE). It is important to point out that all these patients had previously been treated with other immunosuppressive therapies including cyclophosphamide, azathioprine and even steroids, oral prednisone or intravenous methylprednisone.[30,31] Since more than 40 % of cases of PML have been reported in patients with SLE who were only minimally immunosuppressed, SLE itself may be considered to predispose for PML.[31]

Effects of Rituximab in Graves' Disease and Orbitopathy

Over the last five years, the potential efficacy of RTX in active GO has been sought and data derived from either case reports or uncontrolled studies have appeared in the literature. Altogether, the effects of RTX in patients with active GO have been studied in 40 patients (see *Table 1*) and, although the lack of inclusion of randomised and controlled patients in the studies suggests caution in generalising results, data reported show that RTX may significantly affect the inflammatory activity and the severity of GO.

The first evidence of efficacy of RTX in active GO was reported in one patient unresponsive to standard intravenous methylprednisolone therapy.[32] She was euthyroid on methyl mecapto-imidazole (MMI) and had well-controlled type 1 diabetes. The clinical response was characterised by a consistent decrease of the clinical activity scores (CAS) (<3) and improvement of ocular motility, but not of hyperthyroidism. In fact, she had a surprisingly sudden relapse of hyperthyroidism, characterised by a dramatic surge of serum TSH receptor antibodies (TRAb) (>85 IU/L), while being B-cell depleted. She eventually underwent thyroidectomy. RTX induced peripheral B-cell depletion for up to six months after two intravenous doses of 1,000 mg and intra-orbital B and T cell depletion at 10 months (see below). El Fassi et al.[33] treated two women with active GO, also resistant to glucocorticoid therapy, with four weekly doses of RTX of 375 mg/m^2. At eight months after treatment, the CAS had decreased from five and six to one and two, respectively, and soft tissue changes, eye motility and proptosis significantly improved in both patients. In both reports the anti-inflammatory effect of RTX was observed as early as four to six weeks after therapy and persisted without disease relapse and any additional therapy. Unfortunately, case reports cannot tell us whether the disease improvement is due specifically to the drug employed or to spontaneous evolution of the disease's natural course towards inactivation. On the other hand, while patients from these reports were unresponsive to repeated cycles of infusions of high doses methylprednisolone, they did improve rapidly and in a stable and consistent way after only two doses of RTX, suggesting that the drug did impact the active phase of the disease.

Subsequently, Salvi et al.[34] conducted an open study and treated with 1 g RTX twice, with a two-week interval, a group of nine patients with active GO, of whom two had mild GO with only lid signs, and compared with a group of 20 patients treated with the standard intravenous methylprednisolone therapy. All patients responded to RTX therapy compared to 80 % of those treated with steroids (see *Table 2*).

CAS values significantly decreased from 4.7 to 1.8 at the end of follow-up and more rapidly compared with steroids. Proptosis, eye muscle motility and signs of soft tissue inflammation also improved significantly in response to RTX. Relapse of active GO was not observed in patients treated with RTX, but occurred in 10 % of those treated with steroids, who also experienced adverse effects more frequently (45 % versus 33 % of patients) (see *Table 2*).

Table 3: Percentages of Peripheral Lymphocytes at Baseline, at One Hour and at 1–24 Weeks after a Single Small Dose of Rituximab in Three Patients with Active Graves' Orbitopathy

	Time	CD20+	CD19+	CD19+5+	CD3+	CD4+	CD8+
Patient 1	Basal	10.2	9.9	1.4	84.2	62.3	24.1
	45 minutes	0.4	0.7	0.1	92.5	68.5	26
	1 week	0	0	0	84.2	59.5	32.3
	3 weeks	0	0	0	88.6	65	29.1
	6 weeks	0	0	0	90.1	64	28.4
	10 weeks	0	0	0	91.4	67.6	28.2
	13 weeks	0	0	0	90.7	60.7	30.2
Patient 2	Basal	13	12.7	1	81	73.6	9.9
	60 minutes	0	3.2	0.2	88.9	81.9	8.1
	1 week	0	0	0	94.8	87.7	9.4
	3 weeks	0	0	0	93.5	85.8	10.8
	7 weeks	0	0	0	94	83.9	11.8
	11 weeks	0.6	0.6	0.1	92.8	85.5	9.4
	16 weeks	1.9	1.8	0.6	90.7	83.9	10.2
Patient 3	Basal	13.6	13.9	2.1	69.7	44.9	29.9
	45 minutes	0.1	3.1	0.4	69.1	45.8	33.9
	1 week	0.1	0.2	0	82.8	51.5	37.0
	4 weeks	0	0	0	79.4	49.9	40
	8 weeks	0	0	0	76.7	51	35
	16 weeks	1.6	1.6	0.5	82.4	54.3	34.8
	24 weeks	2.2	2.3	0.8	78.3	53.2	30.7

More recent data have confirmed the therapeutic effects of RTX in active GO. Khanna et al.[35] have reported that in six patients with active and severe GO, unresponsive to glucocorticoid therapy, RTX had a rapid and sustained therapeutic effect on both activity and severity. In this study, RTX was also infused intravenously as 1 g twice with a two-week interval, along with steroid therapy. The CAS decreased from 5.5 to 1.8 at eight weeks after RTX and remained low at six months. No patients showed improvement of extra-ocular motility or proptosis, but in four of these patients who had optic neuropathy, visual acuity improved within four weeks and returned to pre-morbid values at eight weeks from treatment. Tapering of glucocorticoids after RTX treatment was not followed by relapse of inflammatory signs. Two patients experienced minor side effects and one had sudden cardiac death later on, unlikely related to such treatment. Another recent open study conducted by Silkiss et al.[36] has shown significant improvement of active GO in 12 patients after RTX administered at the dose of 1 g, two weeks apart. The mean CAS decreased from 5.5 to 1.9 at 16 weeks as well as the mean Thyroid Associated Ophthalmopathy Scale (TAOS), modified by Dolman and Rootman (VISA Classification),[37] from 10.4 to 7.1. Improvement was further recorded up to 52 weeks of follow-up without evidence of relapse of inflammatory signs. Interestingly, no side effects were reported. There has been an unpublished report of an ongoing open study in Newcastle, UK by Mitchell et al.,[38] in which RTX has been administered, even at a lower dose of 500 mg twice, to six patients with active GO refractory to steroid treatment. GO improved fairly rapidly in five patients, with a decrease of the CAS from 5.5 to two at 16 weeks, and remained unchanged in one, without occurrence of side effects. Single patients treated with RTX have also been reported recently. A significant therapeutic effect of RTX was observed[39] in one patient with active GO and stiff person syndrome and diabetes, with complete and persistent inactivation of GO and amelioration of the spastic paresis characteristic of the muscular disease. In contrast to all previous studies, failure of RTX in improving GO but subsequent progression to optic neuropathy was described in one patient,[40] unresponsive to high dose steroids. It is questionable if disease deterioration was caused by the therapy: optic neuropathy in this patient might have been subclinical, due to his unresponsiveness to steroids, and RTX was perhaps given too late to prevent further progression of GO severity. Besides being limited in their number, GO patients treated with RTX so far also had different thyroid status, baseline severity of GO and previous, often unsatisfactory immunosuppressive treatment. A novel treatment for GO is most needed, and only controlled studies will provide evidence on the efficacy and safety of RTX. These studies will also help us in deciding whether RTX is to be used as a first-line therapy in any patients with active GO or only in those with otherwise unresponsive disease of severe degree.

A potentially successful and different approach to this treatment is suggested by a very recent report from Salvi et al. who observed that the doses of RTX currently used in treating GO may be uselessly excessive.[41] For the first time the author found that a low dose of RTX (100 mg) caused effective peripheral B-cell depletion and induced long-term remission of GO, without further treatment. There have been no data previously reported in the literature on the time required to attain total B-cell depletion after RTX infusion in autoimmune disease. This observation was made after discontinuing RTX in two patients because of an important infusion-related reaction. The patients described had mean baseline CAS of 5.3 which decreased to 1.6 at 16 weeks after 100 mg RTX, similarly to what has been reported after treatment with the full dose of 2,000 mg. The amelioration of GO was stable during follow-up. A study employing low dose RTX is now ongoing on a larger group of patients and is aimed at confirming such preliminary unexpected findings, potentially interesting also from the point of view of the safety concerns in using higher doses of a potent immunosuppressive agent like RTX.

The Effect of Rituximab on Hyperthyroidism and Circulating Autoantibodies

One controlled study[42] and two open studies have addressed the effect of RTX on the hyperthyroidism of GD,[34,43] but data are inconsistent. In a controlled study, El Fassi et al.[42] treated ten patients with newly diagnosed and untreated hyperthyroidism with methimazole (MMI) and RTX and 10 with only MMI until they became euthyroid. Within one year of follow-up in all patients treated with MMI alone, but in only six of 10 treated with MMI and RTX hyperthyroidism relapsed. Patients euthyroid at 30 months after RTX had serum TRAb levels not greater than 5 IU/L, which could be predictive of sustained remission. Subsequently, the same authors have reported[44] that RTX treatment in GD patients may favourably affect disease remission by distinctively acting on the TSAb subpopulation with TRAb. In that study, an 84 % decrease in cAMP production by TSH receptor-transfected CHO cells was obtained with sera from patients treated with RTX after 20 weeks, but not with sera from patients treated with MMI. In explaining these findings the author postulated that RTX may affect specifically autoreactive short-lived TSAb-producing plasma cells.[45] Unfortunately, in this study peripheral B cells after RTX therapy were not measured and changes in serum TRAb and other autoantibodies could not be studied in relation to B-cell depletion or return in the peripheral blood.[46] The open study of Salvi et al.[34] mainly addressed the therapeutic potential of RTX in active GO. They nevertheless reported a decrease of serum TRAb levels in both RTX- and glucocorticoid-treated patients with active GO after 30 weeks, but the change was not significantly related to the time elapsed from therapy and did not correlate with either peripheral B-cell depletion or repopulation. Follow-up was at 12 months after RTX in eight patients and five months in one. RTX did not affect thyroid function, since GD patients who were hyperthyroid and untreated showed no improvement in their thyroid function and had to be started on MMI. In a recent follow-up study, Vannucchi et al.[47] could not demonstrate a distinct effect of RTX on serum thyroid stimulating antibody (TSAb) autoantibodies, also measured as immunoglobin (Ig)-stimulation of cAMP by TSH receptor-transfected CHO cells, which appeared to be unchanged and to fluctuate with an identical pattern compared with serum TRAb in either hyperthyroid or euthyroid GD patients. These discrepancies may be due to a too-small number of patients' sera with sufficiently high TRAb levels for an accurate analysis and possibly to the heterogeneity of the clinical characteristics of the patients included in these studies, i.e. thyroid function, duration of disease and presence or absence of GO. Again, larger and controlled studies are needed before drawing significant conclusions. Serum antithyroperoxidase (TPOAb) and antithyroglobulin antibodies (TgAb) were also shown to decline after RTX therapy,[34] but the change was not significant and did not correlated to either B-cell depletion or return in the peripheral blood. Similar data were reported by El Fassi et al.,[42] who were not able to observe a change of serum antithyroid peroxidase antibodies (TPOAb) in relation to treatment with RTX in a series of GD patients, while they did not examined TgAb. Other autoantibodies directed against putative orbital autoantigens, derived mainly from eye muscles[48] have been measured in patients with active GO. No significant change from baseline was observed after RTX therapy in any of the circulating antibodies against the three orbital antigens, calsequestrin, XIII collagen and flavoprotein subunit of succinate dehydrogenase (FP-SDH), respectively.[47] In an uncontrolled study, Heemstra et al.[43] treated 13 patients with relapsing GD, of whom three with mild thyroid-associated ophthalmopathy (TAO) (23 %). On follow-up examination at 26 weeks after RTX, four patients had a relapse of hyperthyroidism despite RTX treatment and received radioiodine therapy, while the remaining nine patients became euthyroid and remained so for a median of 18 months. In this study, serum TRAb levels decreased significantly in nine patients, but did not correlate with B-cell depletion. Consistently with the data of El Fassi et al.,[42] in the GD patients who remained euthyroid, serum TRAb level before RTX therapy were relatively low (median 4 IU/L, range 0.2–6.3). What is not explained by the results of this study is why RTX treatment would have no effect on 31 % of GD patients who were more hyperthyroid, with higher serum thyroid hormone concentrations and TRAb and eventually needed radioiodine therapy. Perhaps the lack of control subjects has prevented the author from providing a more conclusive interpretation of their findings on a potential role of RTX on GD hyperthyroidism remission rates.

Effects of Rituximab on Lymphocytes in the Peripheral Blood and Infiltrating Target Tissues

In patients with GO, data on peripheral B- and T-cell changes after RTX have been reported in the studies of Salvi et al.,[32,34,49] and Heemstra et al.[43] In general, RTX has been shown to induce total peripheral B-cell depletion in most patients.[34,35,43] Interestingly, the clinical response to RTX in both GD[43] and GO patients[34] bore no clear correlation with either CD20+ cell depletion or return in the peripheral blood. When studied, B cells were not detected in the periphery at the time of second dose of 1,000 mg of RTX, at two weeks.[32,34] Peripheral B-cell depletion has generally been reported to last 16–18 weeks. Very recently, we have observed total peripheral depletion of CD20+ and CD19+ cells 45–60 minutes after infusing only 100 mg of RTX in three patients.[41] *Table 3* shows the B-cell depletion pattern in these patients. B-cell depletion lasted 16 weeks, similar to what we observe in patients treated with the full course of RTX therapy at the dose of 1,000 mg x 2 cycles. Typically, no changes were observed in the percentages of peripheral CD3+, CD4+ and CD8+ cells. Although most studies focus on the measurement of peripheral blood B lymphocytes before and after RTX therapy, their analysis might yield findings of limited significance because B cells in the peripheral blood represent <2 % of total mature B cells in the body.[3] In addition, the self-reactive B-cell residing in the bone marrow will not be altered by RTX treatment because B cell receptor selection occurs before CD20 expression.[50]

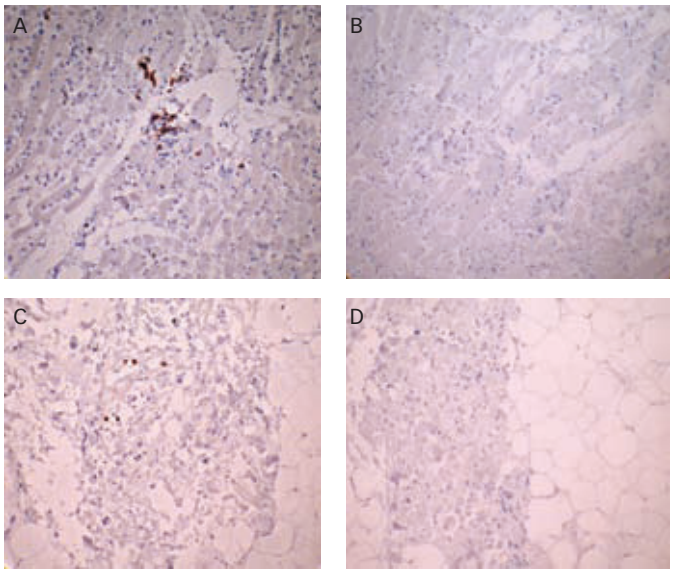
Therefore, in organ-specific autoimmune disease, the therapeutic effect of RTX is likely depending on its interaction with the lymphocytic infiltrates within the target organs, e.g. the thyroid in GD and the orbit in GO. Until now, data on the effect of RTX within target organs in autoimmune disease,[51] including GD and GO, are limited to studies of single case reports. While B cells have been reported to be completely absent from thyroid tissue specimens of one patient with GD one week after RTX therapy,[52] in another CD20+ cells have been shown to be present in the thyroid five months after RTX.[32] This discrepancy might be due to the different time of tissue sampling, since the latter study has been carried out at the time of B-cell return in the peripheral blood. Orbital tissue specimens, usually obtained at surgical decompression, have also been studied in single patients. Orbital tissue depletion of both B and T cells 10 months after RTX was firstly reported by Salvi et al.,[32] and similar findings were recently confirmed as early as 12 days from therapy.[35] Incomplete orbital tissue B-cell (and T-cell) depletion was observed in another two patients studied at approximately six months after RTX therapy,[49,53] both also characterised by incomplete peripheral B-cell depletion. This may

result from long lasting inactivation of autoreactive B cell-induction of inflammation by RTX, since in both patients GO became rapidly inactive and remained so on follow-up. Complete CD20+ cell depletion and either complete or near complete absence of CD3+ lymphocytes in the orbit may depend on the time elapsed from RTX therapy (see *Figure 3*). Of interest, we have recently observed significant infiltration of CD68+ macrophages after either full dose or low dose RTX.[41] After performing further staining for CD1a and CD163, CD1a was found negative, indicating absence of dendritic cells, while CD163, a marker of type 2 macrophages, was well expressed and was particularly abundant in the two patients in whom RTX had been administered more recently.[41] By comparison, control patients only had focal CD68+ and CD163+ cell infiltration. Recruitment of type 2 macrophages might be involved in the mechanism of action of RX in GO and would offer an explanation for its rapid effect. In the work of Khanna et al.,[35] GO improvement and stabilisation after RTX was also associated with the detection of abundance of CD25 cells (T regs) in the peripheral blood, which have been shown to be predictive of RTX therapeutic success in rheumatoid arthritis.[54]

What is B-cell Depletion Showing us in Autoimmune Disease?

Based on the evidence obtained with RTX therapy in both animals and humans, B cells may contribute most significantly to the initiation of disease, as was observed early in the course of diabetes in non-obese diabetic (NOD) mice,[55] in which it was not possible to reverse disease progression once inflammation had begun. This is because B-cell depletion *in vivo* has been shown to significantly decrease autoantigen-specific CD4+ T-cell proliferation in NOD mice, but not to inhibit T-cell expansion once fully initiated.[2] More studies are needed to determine the significance of B-cell depletion therapy in humans by comparison to experimental animal models. B cells, T cells, and autoantibodies are known to be all involved in autoimmune disease pathogenesis, with B cells likely to contribute the most during early disease, while T-cell activation and autoantibody production may independently mediate disease progression.[2] Based on the preliminary observations herein reviewed, an optimal strategy for controlling the progression of GO would be to pursue B-cell depletion shortly after diagnosis and perhaps not as a therapeutic option only when standard immunosuppression has failed. In addition, patients with other autoimmune disease like RA are almost always treated with RTX in combination with standard immunosuppressive drugs. The combination of RTX with other immunosuppressants, e.g. glucocorticoids in GO may more consistently suppress the immune system than B-cell depletion alone, although we do not know if this would bear a greater risk of more severe side effects. While B-cell depletion will affect autoantigen-specific T-cell activation and autoantibody synthesis, immunosuppression will interfere with the clonal expansion of autoreactive lymphocytes and synergistically decrease disease progression. Further understanding of the relative role of B and T cells, as well as autoantibodies, in each autoimmune disease will be critical for optimising future treatment strategies. ■

Figure 3: Immunohistochemistry View of Eye Muscle (A, B) and of Fat Tissue of the Orbit (C, D) of a Patient Treated with Rituximab



A: an interstitial infiltrate of CD3 immunostained T lymphocytes. B: immunostaining for CD20 in an analogous field shows no immunoreactive cells. C: rare CD3 immunostained T lymphocytes infiltrating fibro-fatty tissue. D: immunostaining for CD20 in an analogous field of fibro-fatty tissue shows the complete absence of immunoreactive B cells. All pictures are taken at x200 magnification.

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