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, 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 behind the use of RTX 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 have a significant effect on the inflammatory activity and severity of GO. However, caution is suggested before proposing RTX as a novel therapeutic tool in this disease until randomized controlled trials 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, rather than only as an alternative therapeutic option when standard immunosuppression has failed.

Keywords

Graves' orbitopathy (GO), Graves' disease (GD), B lymphocytes, CD20, CD68, CD163, rituximab, thyroid stimulating hormone receptor antibodies (TRAb), thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb)

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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. In fact, B-cells are involved in multiple pathways of the immune system in autoimmune disease through their combining 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] for example, 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-alpha, 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 an important role in the suppression of autoimmune and inflammatory

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 (at 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 patients improved	3 (minor)	No
El Fassi et al., 2007[42]	2	375 mg/m^2 weekly for 4 weeks	5.5	1.5	All patients improved	1 (minor)	No
Khanna et al., 2010[35]	6	1 g twice with 2-week interval	5.5	1.3	All patients improved	2 (minor) 1 (major, i.e., 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 patients improved	None	No
Mitchell et al., 2010[38]	6	500 mg or 1 g with 2-week interval	5.5	2	5 patients improved 1 patient unchanged	None	Not reported
Krassas et al., 2010[40]	1	1 g twice with 2-week interval	7	7	Patient worsened	Not reported	Yes
Madaschi et al., 2010[39]	1	1 g twice with 2-week interval	5	0	Patient improved	No	No
Salvi et al., 2011[41]	3	Single dose of 100 mg	5.3	1.6	All patients improved	2 (major but transient)	No

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

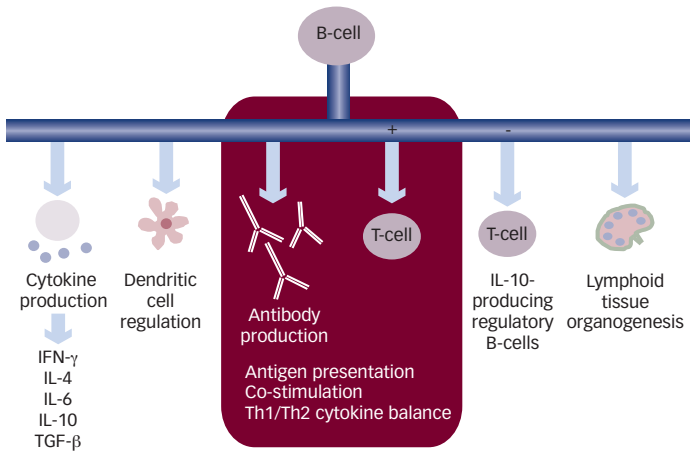
disease. IL-10 produced by B-cells has been shown to downregulate autoreactive immune mechanisms in collagen-induced arthritis[14] and inflammatory bowel disease.[15] As a consequence, B-cell elimination using rituximab (RTX) was in fact associated with exacerbation of ulcerative colitis[16,17] and psoriasis,[18] both being Th1-mediated autoimmune conditions.

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 do not respond to tumour necrosis factor (TNF) inhibitors. In mice, RTX depletes more than 95 % of mature B-cells in blood and primary lymphoid organs within two days of a single dose.

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 or 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-cells and short-lived plasma cells by depletion of 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 (Ig) 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 because it indirectly affects autoantibody production. Consequently, novel

Figure 1: The Multiple Functions of B Lymphocytes



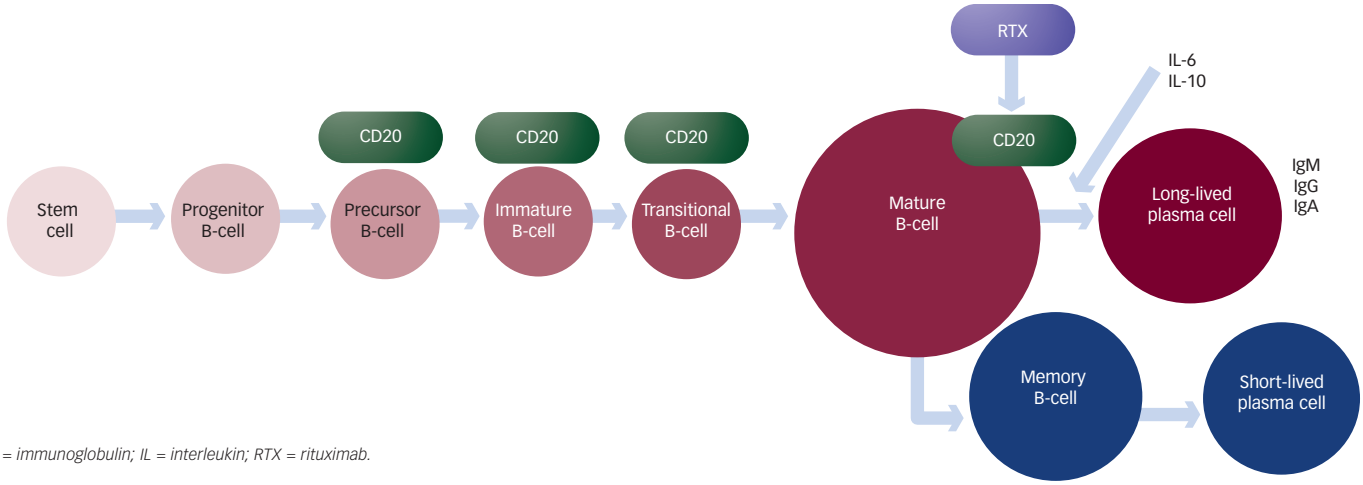
IFN-γ = interferon-gamma; IL = interleukin; TGF-β = transforming growth factor-beta.

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; they have shown that serum concentration of RTX directly correlated with response and inversely correlated with tumor mass.[26] Variable half-lives (11–105 hours) may thus result from the different tumor 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

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 = immunoglobulin; IL = interleukin; RTX = rituximab.

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

		RTX	Glucocorticoid
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]

longer, i.e., up to 20 days after two doses of 1,000 mg (the dose that is 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 costs; should lower doses be as effective as the higher doses that have been employed so far in autoimmune disease based on studies carried out in lymphomas, the cost of treatment would be more affordable by specialised clinical centers.[27]

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 natural killer (NK) cells is the underlying mechanism. Additional mechanisms, such as activation of complement cascade, may be responsible for 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 possible major side effects, progressive multifocal leukoencephalopathy (PML) has been reported in rare cases of 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 to PML.[31]

Effects of Rituximab in Graves' Disease and Graves' Orbitopathy

Over the last five years, the potential efficacy of RTX in active Graves' orbitopathy (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 randomized and controlled patients in these studies means one should be cautious about generalizing results, data reported show that RTX may have a significant effect on the inflammatory activity and 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 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, while being B-cell depleted, the patient had a surprisingly sudden relapse of hyperthyroidism, characterised by a dramatic surge of serum TSHR antibodies (TRAb) (>85 international units[IU]/l). 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 375 mg/m^2 RTX weekly for four weeks. Eight months after treatment, the CAS had decreased from 5 and 6 to 1 and 2, respectively, and soft tissue changes, eye motility, and proptosis had significantly improved in both patients. In both patients, the anti-inflammatory effect of RTX was observed as early as four to six weeks after therapy and persisted without disease relapse or any additional therapy.

Unfortunately, these case reports do not tell us whether the disease improvement was due specifically to the drug employed or to the spontaneous evolution of the disease toward inactivation. However, while patients in these reports were unresponsive to repeated cycles of infusions of high-dose methylprednisolone, they did improve rapidly and

Table 3: Percentages of Peripheral Lymphocytes at Baseline and at Different Follow-up Time-points after a Single Small Dose of Rituximab in Three Patients with Active Graves' Orbitopathy

	Time	CD20+	CD19+	CD19+5+	CD3+	CD4+	CD8+
Patient 1	Baseline	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	Baseline	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	Baseline	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

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 a 1 g dose of RTX repeated after a two-week interval, a group of nine patients with active GO, of whom two had mild GO with only lid signs, and compared it with a group of 20 patients treated with the standard intravenous methylprednisolone therapy (see *Table 2*). All patients responded to RTX therapy, compared with 80 % who responded to the glucocorticoid. With RTX, CAS values had significantly decreased from 4.7 to 1.8 at the end of the follow-up period, and decreased more rapidly than with the glucocorticoid. Proptosis, eye muscle motility, and signs of soft tissue inflammation also improved significantly with RTX. Relapse of active GO was not observed in the patients treated with RTX, but occurred in 10 % of those treated with the glucocorticoid. More patients on the glucocorticoid than on RTX experienced side effects (45 % versus 33 %, respectively).

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 had a rapid and sustained therapeutic effect on both disease activity and severity. In their study, RTX was also given intravenously as a 1 g dose repeated after a two-week interval, along with steroid therapy. The CAS had decreased from 5.5 to 1.8 at eight weeks after treatment and remained low at six months. No patient showed any improvement of extra-ocular motility or proptosis, but, in four patients who had optic neuropathy, visual acuity improved within four weeks of treatment and had returned to pre-morbid values at eight weeks after treatment. Tapering of glucocorticoids after 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 treatment.

Another recent open study, conducted by Silkiss et al.,[36] has shown significant improvement of active GO in 12 patients after administration of one dose of 1 g RTX repeated two weeks later. The mean CAS decreased from 5.5 to 1.9 at 16 weeks, and the mean scores on the Thyroid Associated Ophthalmopathy Scale (TAOS)—as modified by Dolman and Rootman (VISA Classification)[37]—decreased from 10.4 to 7.1. Improvement was further recorded up to 52 weeks after treatment, 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 was administered at 1 g or at an even lower dose of 500 mg (and repeated two weeks later) to six patients with active, steroid-refractory GO. GO improved fairly rapidly in five patients, with a decrease of the CAS from 5.5 to 2 at 16 weeks, and remained unchanged in one, without any occurrence of side effects.

Single case reports of RTX treatment have also been recently published. A significant therapeutic effect of RTX was observed in one patient with active GO, stiff person syndrome, and diabetes, with complete and persistent inactivation of GO and amelioration of the spastic paresis characteristic of the muscular disease.[39] In contrast to all previous studies, failure of RTX in improving GO and subsequent progression to

optic neuropathy was described in one patient who was unresponsive to high-dose steroids.[40] It is questionable whether disease deterioration was caused by the therapy: optic neuropathy might have been subclinical and due to the patient's unresponsiveness to steroids, and RTX was perhaps given too late to prevent further worsening of GO severity.

Besides being limited in number, the population of GO patients treated with RTX so far is also heterogenous in terms of their thyroid status, baseline disease severity and previous (often unsatisfactory) response to immunosuppressive treatment. A novel treatment for GO is most needed, and only controlled studies will provide firm evidence on the efficacy and safety of RTX. These studies will also help us decide whether RTX should be used as first-line therapy in all patients with active GO, or only in those with severe disease unresponsive to other treatments.

A different—and potentially successful—approach is suggested by a very recent report from Salvi et al., who found that the doses of RTX currently used to treat GO may be needlessly excessive.[41] For the first time, it was shown 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 had 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 was observed after RTX was discontinued in two patients because of strong infusion-related reactions. The patients had mean baseline CAS of 5.3, which decreased to 1.6 at 16 weeks after only 100 mg of RTX, similarly to what has been reported after treatment with a total dose of 2,000 mg. During follow-up, the amelioration of GO was stable. A study employing low-dose RTX is now ongoing in a larger group of patients to confirm these unexpected preliminary findings, potentially interesting also from the point of view of the safety concerns arising from the use of high doses of a potent immunosuppressive agent such as RTX.

Effects of Rituximab on Hyperthyroidism and Circulating Autoantibodies

One controlled study[42] and two open studies[34,43] have addressed the effects of RTX on the hyperthyroidism of GD, but data are inconsistent. In the controlled study, El Fassi et al.[42] treated 10 patients suffering from newly diagnosed and untreated hyperthyroidism with methimazole (MMI) and RTX, and 10 with MMI only, until they became euthyroid. Within one year of follow-up, all patients treated with MMI alone, but only six of the 10 patients treated with MMI and RTX, experienced hyperthyroidism relapse. Patients euthyroid at 30 months after MMI and RTX had serum TRAb levels not greater than 5 IU/l, which could be predictive of sustained remission.

Subsequently, El Fassi et al.[44] have reported that RTX treatment in GD patients may favourably affect disease remission by distinctively acting on the thyroid stimulating antibody (TSAb) subpopulation with TRAb. In that study, an 84 % decrease in cyclic adenosine monophosphate (cAMP) production by TSHR-transfected Chinese hamster ovary (CHO) cells was obtained with sera from patients treated with RTX after 20 weeks, but not with sera from patients treated with MMI. When explaining their findings, the author postulated that RTX may specifically affect autoreactive short-lived TSAb-producing plasma cells.[45] Unfortunately, in that study, peripheral B-cells after RTX therapy were not measured, and thus 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 by Salvi et al.[34] mainly addressed the therapeutic potential of RTX in active GO. It nevertheless reported a decrease of serum TRAb levels in both the RTX- and the 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 therapy in eight patients, and at five months in one patient. RTX had no effect on 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 TSAb autoantibodies, also measured as Ig-stimulation of cAMP by TSHR-transfected CHO cells, which appeared to be unchanged and to fluctuate with an identical pattern compared to serum TRAb in either hyperthyroid or euthyroid GD patients. These discrepancies may be due to the sera coming from a number of patients too small for it to contain 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, disease duration, and presence or absence of GO. Again, larger and controlled studies are needed before drawing significant conclusions.

Serum anti-thyroglobulin antibodies (TgAb) and anti-thyroid peroxidase antibodies (TPOAb) were also shown to decline after RTX therapy,[34] but the change was not significant and did not correlate with 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 TPOAb in relation to treatment with RTX in a series of GD patients, while they did not examine 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).[47]

In an uncontrolled study, Heemstra et al.[43] treated 13 patients with relapsing GD, of whom three (23 %) had mild thyroid-associated ophthalmopathy (TAO). 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 that study, serum TRAb levels decreased significantly in nine patients, but did not correlate with B-cell depletion. Consistent with the data from El Fassi et al.,[42] in the GD patients who remained euthyroid, serum TRAb levels before RTX therapy were relatively low (median 4 IU/l, range 0.2–6.3). What is not explained by the results 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 authors from providing a more conclusive interpretation of their findings on a potential role of RTX on GD hyperthyroidism remission rates.

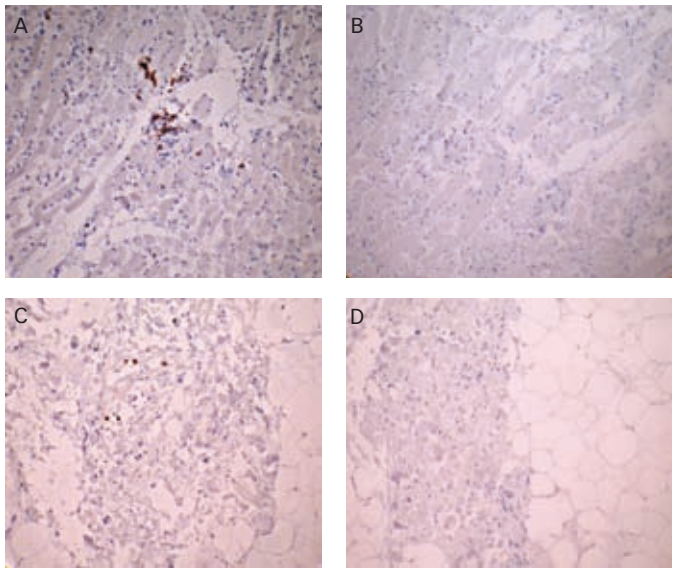
Effects of Rituximab on Lymphocytes in the Peripheral Blood and Infiltrating Target Tissues

In patients with GO, data on peripheral B- and T-cell changes after RTX treatment 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 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 with active GO.[41] *Table 3* shows the B-cell depletion patterns in these three patients. B-cell depletion lasted 16 weeks, similar to what has been observed in patients treated with the full course of RTX therapy (1,000 mg RTX repeated once after a two-week interval). 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-cells 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 the interaction of RTX 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 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 patient, CD20+ cells have been shown to be present in the thyroid five months after RTX therapy.[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 therapy was first reported by Salvi et al.,[32] and similar findings were recently confirmed as early as 12 days after treatment.[35] Incomplete orbital tissue B- 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 at 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*).

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.

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 an 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 RTX 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 an abundance of CD25 cells (T regs) in the peripheral blood, which have been shown to be predictive of RTX therapeutic success in RA.[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 autoimmune 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 with experimental animal models. B-cells, T-cells, and autoantibodies are all known to be 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 reviewed in this article, an optimal strategy for controlling the progression of GO would be to pursue B-cell depletion shortly after diagnosis, and perhaps not use it only when standard immunosuppression has failed. In addition, patients with other autoimmune disease such as 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 of autoantibodies, in each autoimmune disease will be critical for optimising future treatment strategies. ■

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