New immunomodulators in the treatment of Graves’ ophthalmopathy

De nouveaux immunomodulateurs dans le traitement de l’ophtalmopathie basedowienne

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Abstract

Steroids have been used in the therapy of the moderate to severe forms of Graves’ ophthalmopathy (GO) and other autoimmune diseases as they act only as general immunosuppressants. Previous work has shown that blocking the CD-20 receptor on B lymphocytes has significantly affected the clinical course of GO, by rapidly reducing inflammation and the degree of proptosis. We have studied nine patients with Graves’ disease, of whom seven had active GO and two, with newly diagnosed hyperthyroidism, only mild lid signs. We also studied a group of 20 consecutive patients, treated with intravenous glucocorticoids (IVGC) according to a standard protocol. Patients treated with RTX (1000 mg i.v. twice at two-week interval) and those treated with IVGC (500 mg i.v. for 16 weeks) were studied monthly up to 12 months after the first drug infusion. By ophthalmological examination, GO was assessed by the clinical activity score (CAS) and by the NOSPECS score. Thyroid function and lymphocyte count were measured by standardized methods. RTX was well-tolerated and only minor side-effects were reported in 30% of patients during the first infusion. All patients attained peripheral B-cell depletion with the first RTX infusion. All but one patients showed both CD20+ cells and CD19+ cells depletion, while one had persistent 3–5% CD19+ cells in the periphery, mostly CD19 + 5+. Thyroid function was not affected by RTX therapy. Titers of antithyroglobulin (TgAb), antithyroperoxidase and anti-TSH receptor antibodies (TRAb) did not change significantly (P = NS) and did not correlate to CD20+ depletion (P = NS). CAS values decreased significantly (P < 0.0001). Proptosis decreased significantly after RTX in both patients with active GO (ANOVA; P < 0.0001) and in those with Graves’ hyperthyroidism and lid signs (ANOVA; P < 0.003). The degree of inflammation (NOSPECS class 2) decreased significantly in response to RTX (ANOVA; P < 0.001). In patients treated with IVGC, mean CAS value decreased significantly less than in those treated with RTX (P < 0.05). Adverse effects were more frequent after IVGC (45% of patients). Seventy-five percent of patients responded to IVGC and 10% showed relapse of active GO six to eight weeks after withdrawal. The results of this study on RTX in GO suggest that the drug is effective in modifying the disease course and that the improvement of the clinical activity of GO after RTX was more significant than after IVGC. We did not observe relapse of active GO even after B-cell return in peripheral blood. This might be related to the persistence of a significant degree of B-cell depletion after RTX observed in the peripheral blood as late as two years of follow-up. RTX therapy was also effective in improving proptosis and soft tissue inflammation. The mechanism by which RTX affects GO is unknown. It may act as a true immunosuppressor by switching off reactions inducing the active phase of TAO, perhaps by influencing the cytokine production in the orbit or by inducing depletion of antigen presenting B-cells.

Keywords: Thyroid-associated ophthalmopathy; Graves’ disease; Rituximab; B lymphocytes; TSH receptor antibodies; CD 20; CD 19; Immunosuppressive therapy

1. Introduction

Th1 cytokine-driven mechanisms are thought to be involved in the pathogenesis of Graves’ ophthalmopathy (GO) at least in the early stages [1–3]. Recently it has been shown in vitro that TNF-α receptor is not expressed by orbital tissue in the active disease phase [4]. In addition, IL-1 stimulates glycosaminoglycans production and adipogenesis by fibroblasts, but this effect is similarly observed in tissues derived from both GO patients and normal subjects [5]. Taken together these findings question the role of Th1 cytokines in the setting of the disease. Very recently, Douglas et al. [6] have reported that memory T-cells of Graves’ disease patients express IGF-1 receptor that could be the target of auto-antibodies in GO which stimulate fibroblasts growth and...
prolong survival of T-cell, thereby perpetuating the autoimmune process. We have also previously found elevated serum soluble IL-6 receptor (sIL-6R) in active GO, independently of thyroid autoimmune reactions [7]. It is therefore possible that also Th2-mediated mechanisms may be implicated in its pathogenesis.

Thus far, in the therapeutic approach of GO, we have been using agents that act only as general immunosuppressants, also employed in other autoimmune diseases. Steroids have been used for many decades as the mainstay of therapy for the moderate to severe forms of the disease [8]. Evidence derived from clinical studies suggests that immunosuppression may be relevant only as a consequence of chronic treatment, perhaps as long as the duration of the disease’s active inflammatory phase [8,9]. Some authors [10,11] have reported a decrease of serum TRAb levels during the course of treatment with steroids, suggesting a direct effect on the potential immune effector involved in some of the TSH receptor-mediated pathogenic mechanisms of GO [12].

In exploring other potential therapeutic agents, abatacept, which targets the T-cell costimulation pathway CTLA4-B7, has shown to be promising in rheumatoid arthritis [13], whereas IL-10 might inhibit the antigen presenting capacity of monocytes and of T-cell clones proliferation [14]. The data from Douglas et al. [6] raise the possibility of developing an effective drug to block the IGF-1 receptor. In addition, tocilizumab, an anti-IL-6R humanized monoclonal antibody that has been shown to block the IGF-1 receptor or return of CD 20+ lymphocytes in the periphery (Fig. 1). All but one patients showed both CD20+ cells and CD19+ cells depletion, while one had persistent 3–5% CD19+ cells in the periphery, mostly CD19+5+. After four to five months from RTX, B-cells began to repopulate the peripheral blood and were as many as 35 and 66% compared to the pretreatment number at 50 and 100 weeks, respectively. Either peripheral CD 3+ or CD 8+ cells were not affected by RTX treatment (Fig. 2). We observed a progressive decrease in the number of peripheral CD 3+DR+ cells after RTX, which at 40–50 weeks were approximately 25% fewer that before therapy and subsequently normalized at 75 weeks.

Thyroid function was not affected by RTX therapy and hyperthyroid patients required therapy with methimazole. Changes after RTX are shown in Table 1. Titers of antithyroglobulin (TgAb), antithyroperoxidase and anti-TSH receptor antibodies (TRAB) did not change significantly (P = NS) and did not correlate to CD20+ depletion (P = NS). Mean serum TgAb levels decreased at the end of the follow-up period and showed a slightly significant, negative correlation with time (P < 0.04). Serum TRAb levels changes did not correlate with either depletion or return of CD 20+ lymphocytes in the periphery (P = NS) and decreased at 50 weeks of follow-up (P < 0.01), when all patients reached stable euthyroidism. CAS values before therapy were 4.7 ± 0.5 and decreased to 1.8 ± 0.8 at the end of follow-up.

2. Study report

We have studied nine patients with Graves’ disease, seven women and two men, aged 31–51 years, of whom seven had active GO and two, with newly diagnosed hyperthyroidism, only mild lid signs. We also decided to study a group of 20 consecutive patients, 17 women and three men, aged 30–82 years, treated with intravenous glucocorticoids (IVGC) according to a standard protocol. Patients treated with RTX (1000 mg i.v. twice at two-week interval) and those treated with IVGC (500 mg i.v. for 16 weeks) were studied monthly up to 12 months after the first drug infusion. By ophthalmological examination, GO was assessed by the clinical activity score (CAS), whereas severity was classified by the NOSPECS score. Thyroid function and lymphocyte count were measured by standardized methods.

RTX was well-tolerated and only minor side-effects were reported in three patients during the first infusion. All patients attained peripheral B-cell depletion with the first RTX infusion which lasted four to five months (Fig. 1). All but one patients showed both CD20+ cells and CD19+ cells depletion, while one had persistent 3–5% CD19+ cells in the periphery, mostly CD19+5+. After four to five months from RTX, B-cells began to repopulate the peripheral blood and were as many as 35 and 66% compared to the pretreatment number at 50 and 100 weeks, respectively. Either peripheral CD 3+ or CD 8+ cells were not affected by RTX treatment (Fig. 2). We observed a progressive decrease in the number of peripheral CD 3+DR+ cells after RTX, which at 40–50 weeks were approximately 25% fewer that before therapy and subsequently normalized at 75 weeks.

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Fig. 1. Effects of rituximab (RTX) on peripheral B lymphocytes in patients with Graves’ ophthalmopathy. Percentages in the circles are referred to basal B lymphocyte count before RTX. CD 20+ cells: CD 19+ cells: CD 20+ cells: CD 19+ cells:
Table 1

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<td>CD 20 depletion vs hyperthyroidism</td>
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<td>Proptosis decrease</td>
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(P < 0.0001). Proptosis decreased significantly after RTX in both patients with active GO (ANOVA; P < 0.0001) and in those with Graves’ hyperthyroidism and lid signs (ANOVA; P < 0.003). The degree of inflammation (NOSPECS class 2) decreased significantly in response to RTX (ANOVA; P < 0.001). We have not observed relapse of active GO in any of the RTX treated patient. In patients treated with IVGC, mean CAS value decreased significantly less than in those treated with RTX (P < 0.05). Adverse effects were more frequent after IVGC (45% vs 33% of patients). Seventy-five percent of patients responded to IVGC and 10% showed relapse of active GO six to eight weeks after withdrawal.

3. Discussion

The results of this study on RTX in GO [20] suggest that the drug is effective in modifying the disease course and its clinical impact. The improvement of the clinical activity of GO after RTX was more significant than after IVGC and seemed to appear relatively earlier. Interestingly, the CAS continued to decline in patients treated with RTX even after the first five months of follow-up, when B-cells returned, whereas the same was not observed in patients after IVGC, some of whom showed, indeed, relapse of active GO. We did not observe relapse of active GO, which was absent at any time even after B-cell return in peripheral blood. This might in part be related to the persistence of a significant degree of B-cell depletion after RTX observed in the peripheral blood as late as two years of follow-up. RTX therapy was also as effective as IVGC in modifying disease severity, as shown by the significant improvement of proptosis and soft tissue inflammation. Interestingly, we reported some differences in the rate of adverse effects, more frequent and bothersome for patients during IVGC than RTX. RTX did not expose patients to opportunistic infections and did not alter their perception of being in good health.

Response to therapy rapidly followed peripheral CD20+ depletion, which was attained in all patients already with the first RTX dose. One patient showed no CD20+ cells after RTX treatment, but persistence of about 3–5% CD19+ cells, of which about 90% were coexpressing CD19+5+, characteristic of auto-reactive clones [21]. This patient was affected by subclinical optic neuropathy which promptly improved after RTX and subsequently relapsed, perhaps due to incomplete intraorbital B-cell depletion. We have also observed an increased prevalence of CD 19+5+ (about 50%) in the intrathyroidal lymphocytes of one patient who underwent thyroidectomy at the time of initial peripheral B-cell return, five months after RTX treatment [18] and had recurrent hyperthyroidism. This lymphocyte subset typically represents cells that initially reconstitute in the peripheral blood [22], as was reported in human systemic lupus erythematosus [23]. Interestingly, peripheral B-cell depletion did not cause changes in circulating serum thyroid auto-antibodies. It seems unlikely that persistent plasmacells, which do not express CD 20, may continue to produce antibodies [16,17], since they would only be measured in the circulation for no longer than four to six weeks [24]. Continuous auto-antibodies production may derive from lymphocytes in the thyroid or in other lymphoid organs such the bone marrow or the spleen [24]: persistence of small germinal centers were in fact shown in thyroid surgical specimens from the patient who underwent thyroidectomy at the time of initial B-cell return [18]. This may also explain why RTX treatment had no effect on hyperthyroidism in Graves’ patients, probably consequent to its absent effect on TRAb production.

The mechanism by which RTX affects GO is at present unknown. It may act as a true immunosuppressor by switching off reactions inducing the active phase of TAO, perhaps by influencing the cytokine production in the orbit [2,7,25]. Since we have observed at histopathology in one patient the absence of lym-
phocytic infiltration in the orbit after RTX treatment, but not in
the thyroid [18], we believe that therapy may induce depletion of
oribital lymphocytes without affecting the intrathyroidal lymphocytic population. Interestingly, in the same patient we did not find either B or T lymphocytes in the orbital tissues, perhaps as a result RTX induced depletion of antigen presenting B-cells, as initiators of orbital autoimmune reactions [25,26]. The observation of a progressive decrease of peripheral CD 3+DR+ cells until about 50 weeks from treatment might suggest in fact reduced antigen presentation in relation to the clinical therapeutic outcome.

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