New immunomodulators in the treatment of Graves’ ophthalmopathy

De nouveaux immunomodulateurs dans le traitement de l’ophtalmopathie basedowienne

M. Salvi a,∗, G. Vannucchi a, I. Campi a, N. Currò b, P. Beck-Peccoza

a Department of Medical Sciences, Endocrinology Unit, University of Milan, 20100 Milan, Italy
b Department of Ophthalmology, University of Milan, 20100 Milan, Italy

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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 (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 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

∗ Corresponding author.
E-mail address: mariosalvi@mariosalvinet.191.it (M. Salvi).
prolonging 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 be a promising agent in rheumatic diseases because of its effect on the blockade of the proinflammatory cytokine milieu [15] might also be effective. Rituximab (RTX) is a humanized chimeric anti-CD20 monoclonal antibody whose variable (antigen-binding) region is derived from a mouse antibody. The binding of RTX to CD20 antigen blocks the activation and differentiation of B-cells, since CD20 protein is expressed on the surface of pre-B and mature B lymphocytes, but not on stem cells, pro-B lymphocytes and plasma cells [16,17]. Therefore, treatment with RTX leads to specific elimination of B-cells without affecting the regeneration of B-cells from stem cells and the production of immunoglobulins by plasma cells. Preliminary work from our laboratory and others has shown that blocking the CD-20 receptor on B lymphocytes has significantly and positively affected the clinical course of the disease, by rapidly reducing inflammation and the degree of proptosis [18,19].

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 than 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.
Table 1

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<td>TgAb titers decrease</td>
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<td>CD 20 depletion vs hyperthyroidism</td>
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<td>CAS decrease</td>
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<td>NOSPECS class 2 improvement</td>
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<td>Proptosis decrease</td>
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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. Since we have observed at histopathology in one patient the absence 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 orbital 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 initiators of orbital autoimmune reactions [25,26]. The observation of a progressive decrease of peripheral CD3+DR+ cells until about 50 weeks from treatment might suggest in fact reduction of a progressive decrease of peripheral CD3+DR+ cells as initiators of orbital autoimmune reactions [25,26]. The observation of a progressive decrease of peripheral CD3+DR+ cells until about 50 weeks from treatment might suggest in fact reduction of a progressive decrease of peripheral CD3+DR+ cells as initiators of orbital autoimmune reactions [25,26].

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