Hodgkins lymphoma. It has subsequently been successfully used in the treatment of various autoimmune diseases, including idiopathic thrombocytopenic purpura, systemic lupus erythematosus, haemolytic anaemia and rheumatoid arthritis. Rituximab causes an immediate depletion of circulating B cells. This depletion lasts for four to six months, thereby interfering with T-cell activities.

Current therapies of Graves’ disease are aimed at reducing thyroid hormone synthesis and consist of thionamides, radioiodine and surgery. Thionamides block the thyroid hormone synthesis by inhibition of thyroid peroxidase, and are usually prescribed for at least one year. The major disadvantage of thionamides is the low remission rate of only 30-40% of patients after 10 years. In addition, rare but serious side effects of thionamides such as agranulocytosis and hepatotoxicity, limit prolonged use of these drugs. Radioiodine therapy is indicated for relapsing Graves’ disease in Europe and Japan, whereas this is the treatment of choice in the US. Hypothyroidism is the major complication of radioiodine therapy with an estimated incidence of 30% in the first two years after therapy and thereafter a yearly incidence of 5%. Surgery is not used often as a treatment for Graves’ disease and is mainly restricted to patients with obstructive goitre, ophthalmopty and uncertain histology of nodes and patients refusing radioiodine therapy. Complications of surgery are lesions of the recurrent and hypopharynx. Obviously, current therapies are not aimed at the underlying pathogenetic mechanisms in Graves’ disease. The imperfections of current therapies for Graves’ disease substantiate the need for new treatment options aimed at the pathology of Graves’ disease.

**Rituximab**

Auto-antibodies directed against the TSH receptor are crucial in the pathogenesis of Graves’ disease. These antibodies are synthesised by infiltrating B cells into the thyroid. Plasma cells from the bone marrow and cervical lymph nodes are also involved in the synthesis of autoantibodies. Therefore, a promising strategy for treating Graves’ disease would be the elimination of activated B-lymphocytes. Pre-B-lymphocytes as well as activated mature B-lymphocytes express CD20 on the surface, but this expression is lost following differentiation into plasma cells. CD20 is also expressed on some pro-B-cells, the precursors of pre-B-cells, although in low numbers. This makes the CD20 antigen an attractive target for treating Graves’ disease. Moreover, anti-CD20 therapy could also interfere with the B-cell antigen-presenting role to T cells and thereby interfere with T-cell activities.

Rituximab is a chimeric monoclonal antibody specific for human CD20. Rituximab was originally used for the treatment of non-Hodgkins lymphoma. It has subsequently been successfully used in the treatment of various autoimmune diseases, including idiopathic thrombocytopenic purpura, systemic lupus erythematosus, haemolytic anaemia and rheumatoid arthritis. Rituximab causes an immediate depletion of circulating B cells. This depletion lasts for four to six months, but may last for more than 24 months. Pre-treatment levels of B cells will be reached after nine to 12 months. Rituximab kills B cells by induction of apoptosis by altering calcium influx, antibody-dependent cellular toxicity and complement-dependent cellular cytotoxicity.

**Graves’ Hyperthyroidism**

Several studies have investigated the effect of treatment of Graves’ disease with rituximab. Infusions with rituximab resulted in a decrease in CD20+ B cells in all patients. Salvi et al. treated nine patients with Graves’ disease with two infusions of rituximab 1g at a two-week interval. At baseline, four patients were hyperthyroid and five were euthyroid (two were on methimazole treatment, two were in remission and one was receiving thyroxin treatment after previous thyroidectomy). Of the four patients who were hyperthyroid at baseline, three had persistent hyperthyroidism after infusions with rituximab. These patients received methimazole. One of the hyperthyroid patients had subclinical hyperthyroidism and became euthyroid eight months after treatment with rituximab. The euthyroid patients without medical treatment remained in remission. In one patient who had received methimazole, hyperthyroidism developed rapidly after discontinuation of methimazole, despite B-cell depletion. Therefore, Salvi et al. concluded that thyroid function is not affected by treatment with rituximab.

El Fassi et al. performed a prospective controlled trial in 20 patients. Most patients had a first episode of Graves’ disease. Ten patients were given four infusions with 375mg/m² of rituximab with a weekly interval, whereas the other 10 received no rituximab. All patients were treated with methimazole.
Thyroid Disorders

until the last infusion with rituximab. Methimazole treatment was thereafter discontinued in all patients. In the 10 patients who were treated with the combination of rituximab and methimazole, six relapsed within one month after discontinuation. In the 10 patients who were treated with methimazole alone, eight relapsed within one month, one within three months and one after 13 months. However, methimazole treatment in this study lasted for around four months, which is unusually short. Therefore, it is not clear whether rituximab would be more efficacious compared with a full course of methimazole therapy.

Heemstra et al. studied 13 patients with relapsing Graves’ disease. All patients were treated with two infusions of rituximab 1g with a two-week interval. Four patients did not respond to treatment with rituximab, whereas free T4 (FT4) levels decreased and TSH levels increased in nine. Median follow-up was 18 months (range 14–20 months). Non-responders had significantly higher FT4 levels than responders. El Fassi et al. found no differences in FT4 levels between responders and non-responders. However, in that study patients were treated with methimazole before treatment with rituximab, whereas in the study of Heemstra et al. methimazole treatment had to be discontinued at least four weeks prior to treatment with rituximab to verify the existence of hyperthyroidism. El Fassi et al. found also a significant difference in thyrotropin-binding inhibiting immunoglobulin (TBI) levels between responders and non-responders, with patients with lower TBI levels responding more favourably to rituximab. In addition, in the study by Heemstra et al. it seems that two non-responding patients had higher TBI levels than responders. It would be useful to study this further to define a subgroup of patients likely to be responders.

Auto-antibodies

Controversy exists about the effect of rituximab on TBI levels. No studies found a relationship between proportions of CD-20+ lymphocytes after rituximab treatment and TBI levels. Salvi et al. observed no significant differences in TBI levels after infusion with rituximab, whereas Heemstra et al. found a significant decrease in TBI levels after treatment with rituximab. This decrease was observed only in the responders. El Fassi et al. also found a decrease in TBI levels after rituximab treatment. However, this decrease was not different to that seen in patients treated with methimazole alone. However, they did show a decrease in the TSH receptor stimulatory capacity of TBI with no change in absolute TBI levels in patients treated with rituximab and methimazole compared with patients treated with methimazole alone, suggesting an alteration of the balance from stimulating into non-stimulating TBI due to decreased production of stimulating TBI. The association between circulating levels of CD20+ lymphocytes, serological markers of autoimmune disease and effectiveness of rituximab in autoimmune disease is complex; however, several studies in patients with rheumatoid arthritis and lupus imply an inverse relationship between proportions of B cells, autoantibody titers and therapeutic effect.

Explanations for a Lack of Effect of Rituximab

Synthesis of new antibodies may occur after rituximab treatment given that the half-life of human immunoglobulin G (IgG) is approximately three weeks. In addition, it has been reported that the levels of immunoglobulin A and G remain unaltered after treatment with rituximab. Another explanation may be that plasma cells that do not express CD20 are an important source of auto-antibodies. Moreover, a portion of plasma cells have a very slow turnover. Furthermore, lymphoid cells in germinal centers in the thyroid may exhibit less pronounced B-cell depletion. However, El Fassi et al. showed complete intrathyroidal B-lymphocyte depletion after treatment with rituximab.

Graves’ Ophthalmopathy

Graves’ disease is also associated with extrathyroidal complications, of which Graves’ ophthalmopathy (GO) is the most prevalent. GO involves inflammation and swelling of retrobulbar fibrous and adipose tissue and extraocular muscles, causing mild to severe symptoms that include proptosis and compression of the optic nerve, affecting quality of life. The pathogenesis of GO is not completely understood, but involves an immunological cross-reaction between antigens of the thyroid and orbital tissues, and includes pathological hyperactivation of orbital fibroblasts, deposition of collagen and glycosaminoglycans in the extracellular matrix and, eventually, fibrosis. T cells also play a role in the pathogenesis of GO. A product of activated T cells, leukoregulin, stimulates the production of hyaluronan, prostaglandin-endoperoxidase H and proteins produced by orbital and pre-tibial fibroblasts.
In patients with Graves’ disease treated with rituximab, mild infusion related reactions occurred in five out of 10 patients and three out of nine patients. In the study by Heemstra et al., two patients developed a serum-sickness-like reaction with joint pain and fever. In the latter, treatment with rituximab was discontinued in two patients. El Fassi et al. also reported the occurrence of ulcerative colitis shortly after treatment with rituximab in a patient with irritable bowel syndrome.

Concluding Remarks

We conclude that rituximab is probably effective in improving GO in patients who are unresponsive to treatment with glucocorticosteroids. However, the results in these patients are less clear and efficacy should be confirmed with a randomised controlled trial. The design of such a study is nevertheless complex, because many confounding factors influence the outcome of these patients. Complex issues in such trial are whether or not to pre-treat patients with threoyestatica drugs and the problem of blinding in studies involving radioiodine therapy. Nevertheless, the introduction of new classes of immunomodulatory drugs such as rituximab offer exciting new perspectives in the development of new approaches for the treatment of Graves’ disease.

Table 1: Overview of the Literature on the Use of Rituximab in Graves’ Disease

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Treatment</th>
<th>Design</th>
<th>Outcome Thyroid Function</th>
<th>Outcome Graves’ Ophthalmopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Fassi et al.32</td>
<td>9</td>
<td>2 infusions of rituximab 1g</td>
<td>Prospective study</td>
<td>No effect on thyroid function</td>
<td>CAS, NOSPECS class 2, NOSPECS class 4 and proptosis improved</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with 2-week interval</td>
<td></td>
<td></td>
<td>CAS more pronounced and earlier improvement in rituximab group compared with glucocorticosteroids group</td>
</tr>
<tr>
<td>El Fassi et al.33</td>
<td>10</td>
<td>375mg/m² rituximab weekly</td>
<td>Prospective controlled study</td>
<td>4 patients remained in remission in rituximab group</td>
<td>Only 2 patients with GO (47): CAS improved</td>
</tr>
<tr>
<td></td>
<td></td>
<td>for 4 weeks and 2 months methimazole, discontinued at last RTX infusion</td>
<td></td>
<td>All patients relapsed in methimazole-alone group</td>
<td>Prophtosis improved in one patient in both eyes and in 1 patient only in the right eye</td>
</tr>
</tbody>
</table>

CAS = clinical activity score; GO = Graves’ ophthalmopathy.