Intralesional Therapy With Anti-CD20 Monoclonal Antibody Rituximab in Primary Cutaneous B-Cell Lymphoma

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Background: We report the use of a new treatment modality in 2 patients with primary cutaneous B-cell lymphoma. In a 58-year-old woman with progressive nodular lesions on the scalp and face, several treatment attempts either failed or could not be used because of severe adverse effects and underlying epilepsy. The patient declined radiotherapy. A 30-year-old man presented with recurrence of tumor nodules occipitally, thoracically, on the arm, and on the right thigh after several excisions.

Observations: Intralesional injection of rituximab, a chimeric antibody directed against the CD20 transmembrane antigen present in malignant and normal B cells, resulted in partial regression of tumor nodules. No adverse effects occurred except pain during or shortly after injection and, in one patient, a slight rise in body temperature. Due to the treatment a prolonged complete disappearance of B cells from peripheral blood samples was observed.

Conclusion: Intralesional rituximab therapy is a nontoxic and effective treatment for cutaneous B-cell lymphoma that deserves further investigation in larger clinical trials.

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Primary cutaneous B-cell lymphomas (CBCLs) are a heterogeneous group of B-lymphoproliferative diseases characterized by restriction to the skin, long-term survival of the patients, and a high incidence of local recurrence after various treatment modalities, including surgical excision, radiotherapy, treatment with interferon alfa, psoralen–UV-A, or chemotherapy.1-4 The neoplastic B cells express B-cell-associated antigens CD19, CD22, CD79a, and CD20. CD20 is a nonglycosylated phosphoprotein (MW 33,35 or 37 kDa) present in malignant B cells, normal mature B cells, and pre-B cells.5 In malignant B cells it is expressed at high surface densities. Hematopoetic stem cells, pro-B cells, and plasma cells are negative for CD20. This transmembrane protein has a physiological function in B-cell differentiation and proliferation, which is likely mediated by a Ca²⁺ channel.6

We report, for the first time to our knowledge, the use of intralesional injection of an anti-CD20 monoclonal antibody as a new treatment modality in a 58-year-old woman who presented with long-standing primary CBCL and exhibited progressive tumor growth on the scalp and face. Chimeric anti-CD20 monoclonal antibodies have been used in the treatment of low-, intermediate-, and high-grade B-cell non-Hodgkin lymphoma as intravenous infusion therapy and are a registered treatment for this indication and application mode in several countries. The use of rituximab was efficient as a monotherapy, as well as in combination with interferon alfa or polychemotherapy.7,8

In a study9 with 4 weekly infusions of 375 mg/m², systemic adverse effects were seen in 86.5% of the patients: fever (73.0%), nausea (18.9%), thrombocytopenia (18.9%), and hypotension (16.2%). Because the lesions in our patients were well circumscribed and confined to a limited area of the body and because of the lack of other applicable treatment modalities, we performed intralesional therapy with the specific anti-CD20 chimeric antibody rituximab. Our treatment modality resulted in favorable clinical effects without clinical systemic adverse effects.

CASE 1

Clinical History

A 58-year-old woman presented in February 1991 with round, red nodules on the
scalp that were painful to palpation and partial alopecia. She had no fever, weight loss, or night sweats. The results of chest radiography, ultrasonography of the abdomen, computed tomography of the head, positron emission tomography,\textsuperscript{10} and a bone marrow biopsy did not reveal additional disease manifestation apart from the cutaneous lesions. Results of the histological examination performed in April 1993 revealed a follicular CBCL. The tumor was resistant to therapy with topical steroids, photodynamic therapy, psoralen–UV-A, and topical carmustine between 1993 and 1997. The injection of cisplatin collagen gel resulted in necrotic tumor debulking with scar formation, but this treatment had to be discontinued because of serious allergic adverse effects, such as urticaria and nausea, which increased with each subsequent injection.\textsuperscript{11} The patient declined radiotherapy since she feared the concomitant hair loss and relapses. Therapy with interferon alfa was considered but not used because the patient had epilepsy, and fever-induced convulsions were expected.

In April 1998 she presented with tumor nodules in the centroparietal region and new lesions on the forehead, which were aesthetically unacceptable to the patient.

**Histological Examination**

We performed a punch biopsy, the results of which revealed a diffuse infiltrate of large CD20\textsuperscript{+}, CD79a\textsuperscript{+}, bcl-2\textsuperscript{+}
cells accompanied by a network of CD21+ dendritic cells and scattered CD4+ and CD8+ T lymphocytes compatible with large cell B-cell lymphoma.

**Therapeutic Approach**

After informed consent was obtained, rituximab was injected 3 times a week; each time, 3 mL of the stem solution (at 10 mg/mL) was injected into the stigmatizing nodules on the face and painful nodules on the scalp followed by a 3-week treatment-free period. Injections were painful because of the pressure created by the additional volume. They were, however, tolerated without local anesthesia. An inflammatory response in the injected lesions was observed approximately 3 hours after each injection and lasted for a couple of hours. This therapy was repeated 6 times every 28 days. After 9 injections, some of the nodules flattened and diminished in size and others had completely resolved (Figure 1).

**Assessment**

The results of blood analysis, which was performed at least monthly during the 6 months after therapy, showed that CD20+ and CD19+ B lymphocytes were completely depleted in the peripheral blood while other lymphocyte subpopulations were not affected, thus verifying the specific systemic effect of rituximab (Figure 2, A and B). No changes were observed in levels of neopterin, β2-microglobulin, or immunoglobulins (Figure 2, C and D). The values for liver enzymes, blood urea nitrogen, and creatinine remained unchanged. The patient did not experience any systemic adverse effects.

Intratumoral injection of this anti-CD20 monoclonal antibody was effective and safe. After a total of 18 injections, another punch biopsy specimen was obtained from one of the injected nodules. Histological results revealed medium to large cell lymphoma. The B cells positive for CD20 and bcl-2 were still present in a biopsy specimen from one of the remaining nodules. The follow-up period was 12 months.

**CASE 2**

**Clinical History**

A 30-year old man had primary CBCL since 1993 with nodules on his back and arms. In August 1996, when he was admitted to our clinic, the results of histological examination showed a follicular CBCL. He had no fever, weight loss, or night sweats. The results of chest radiography, ultrasonography of the abdomen, computed tomography of the thorax and abdomen, and a bone marrow biopsy did not reveal additional disease manifestation. Recurrence of nodules...
lar lesions after various excisions, pain, itching of several nodules, and spontaneous bleeding from one node disturbed the patient. The man declined radiotherapy. In November 1998 he presented for intralesional therapy with anti-CD20 monoclonal antibody since new nodules on his right thigh, occipital and thoracic regions, and on the right side of the abdomen had developed.

**Histological Examination**

The biopsy specimen showed an infiltrate of small CD20+ and CD79a+ cells grouped in follicles accompanied by a network of CD21+ dendritic cells compatible with follicular CBCL.

**Therapeutic Approach**

After informed consent was obtained, treatment was performed as described in patient 1. In patient 2, 2 to 3 hours after injection all nodules showed an inflammatory reaction with erythema, itching, and a slight rise in body temperature that lasted for about an hour. After 6 injections, some of the nodules had softened, diminished in size, and others, including some that were not treated, had completely resolved.

**Assessment**

The results of blood analysis, which was performed monthly during therapy, showed that CD20+ and CD19+ B lymphocytes were selectively depleted while other lymphocyte subpopulations were not affected. No changes were observed in levels of neopterin, β₂-microglobulin, or immunoglobulins. The values for liver enzymes, blood urea nitrogen, and creatinine remained unchanged. The patient experienced a slight rise in body temperature of 0.5°C.

After 6 injections, another biopsy specimen was obtained from one of the remaining injected nodules. Histological results showed follicular CBCL. The administered antibody therapy was effective and safe. During follow-up a new lesion was observed that disappeared with subsequent treatment.

**COMMENT**

Patients with primary CBCLs present with well-circumscribed lesions restricted to one area of the body and have a 5-year survival rate of 67% to 97%. Long-standing relapsing CBCL frequently is not curable. In these cases, therapy has to offer palliation at minimal risk and have little impact on the quality of life. Radiotherapy is the first choice in the treatment of most CBCLs. Systemic chemotherapy is required in a small minority of patients, and interferon alfa can be considered as an alternative drug.

To our knowledge, we report for the first time the intralesional treatment of CBCL with the chimeric anti-CD20 monoclonal antibody, rituximab. This treatment modality was effective, well tolerated, and economical since the applied dose corresponds to only 12% of the dose administered intravenously.

Rituximab is a monoclonal antibody consisting of the variable region of murine anti-CD20 and the constant region of human IgG1 heavy chains and κ light chains. A major limitation of the use of antibodies is their immunogenicity, which results in an immune response consisting of the formation of, for example, human antimouse antibodies, which neutralize the applied antibodies. Effector mechanisms, such as complement-mediated cytotoxicity and antibody-dependent cellular cytotoxicity, are not effectively activated by most murine antibodies in humans. As the major part of rituximab is coded by human exons, it is less immunogenic. At the same time it keeps its biological effector function. The mouse variable regions in the Fab part of each antibody molecule bind to CD20 antigen with high affinity (5 × 10⁻⁹ mol/L) and upon binding mediate a signal to induce cell-cycle arrest and/or apoptosis. Therefore, an antiproliferative effect against malignant B-cell lines also was observed independently of the immune system in vitro. The antibodies are not internalized upon binding, thus B cells are labeled in the tumor nodules and in the peripheral blood. Therefore, in vivo, in addition to the direct antiproliferative and apoptotic effect described above, the human Fc part of the IgG is recognized by the immune system of the patient, inducing 3 pathways leading to elimination of the tumor cells. First, by binding of the first component of the classic pathway of the complement system (C1) to the Fc part of the antibody, complement-mediated cell lysis is triggered. Second, phagocytosis by mononuclear phagocytes and neutrophils occurs upon binding of the Fc part to the IgG receptor FcyR of these effector cells. Third, by recognition of the Fc part through FcyR on natural killer cells, they will be activated and predominantly kill antibody-labeled cells. For the antibody-dependent cellular cytotoxicity IgG1, the subclass of rituximab, and IgG3 are the subclasses that will induce the most efficient response. In the treatment regimens in which rituximab was administered in combination with chemotherapy, the cytotoxic effects of the chemotherapy upon tumor cells were further enhanced.

In patient 1, the tumor was resistant to treatment with local steroids, photodynamic therapy, psoralene–UV-A, and local therapy with carmustine. Injections of cisplatin collagen gel had to be discontinued because of the systemic adverse effects. We achieved a favorable clinical response with the use of rituximab. The stigmatizing lesions on the face disappeared during the course of the treatment. The patient tolerated the procedures well. There were no severe adverse effects. Local administration resulted in a complete depletion of B lymphocytes in the peripheral blood. Our patient had no infections during treatment and follow-up. In a previous study, depleted B cells were replaced 6 months after termination of the therapy and normal values were recorded 9 and 12 months after cessation of the antibody therapy. Despite this depletion, the rate of infection was not elevated and serum immunoglobulin levels were only minimally changed, possibly because of plasma cells that were not depleted by the treatment.

Although the clinical response was impressive, some nodules did not disappear completely. However, patient 1 did not show optimal preconditions since she presented with large cell lymphoma. In previous studies,
the symptoms in patients presenting with follicular histological findings were more likely to show a complete response. Furthermore, the reason for the persistence of some of the injected nodules might be found in the high expression of the apoptosis inhibitor bcl-2, which is rarely observed in primary cutaneous B-cell lymphomas. Staging, however, did not reveal extracutaneous manifestations. Another reason for the limitation in the disappearance of the lesions could be the insufficient infiltration with macrophages and other effector cells that are able to clear the site of antibody-bound B cells. Nevertheless, especially patient 1 was satisfied with the clinical result.

We conclude that intralesional rituximab might be an effective and feasible treatment alternative to systemic intravenous therapy with the anti-CD20 monoclonal antibody. If other treatment options are exhausted or not applicable, the intratumoral injection of rituximab may be a promising treatment approach that deserves further investigation in controlled clinical trials.

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REFERENCES


Correction

Error in Dosage. In the correspondence titled “Ivermectin: A Few Caveats Are Warranted Before Initiating Therapy for Scabies,” published in the December issue of the ARCHIVES (1999;135:1549-1550), all dosage units are in milligrams per kilogram and should have been micrograms per kilogram. Therefore, the following corrections should be made: “200 mg/kg” should be “200 µg/kg,” “250 mg/kg” should be “250 µg/kg,” and “400 mg/kg” should be “400 µg/kg.”