Giant cell hepatitis with autoimmune hemolytic anemia (GCH-AHA) is a rare autoimmune disease of infancy characterized by severe liver disease associated with Coombs-positive hemolytic anemia. We recently showed that GCH-AHA is probably caused by a humoral immune mechanism. Such data support the use of rituximab, an anti-CD-20 monoclonal antibody specifically targeting B lymphocytes, as a treatment for GCH-AHA. We describe here the detailed clinical evolution of 4 children with GCH-AHA who showed a complete response to rituximab. All patients shared a severe course of the disease with poor control on standard and aggressive immunosuppression. Rituximab was well tolerated, and no side effects or infections were registered. Several doses were needed to induce remission, and 5 to 11 additional maintenance injections were necessary in the 2 more severe cases. Weaning from corticosteroids was achieved in all subjects. A steroid-sparing effect was noted in the 3 children who started rituximab early in the course of the disease. Overall, we show that there is a strong rationale for treating GCH-AHA with rituximab. Early treatment could reduce the use of corticosteroids. Nevertheless, short-term steroids should be initially associated with rituximab to account for autoantibodies’ half-life. Repeated injections are needed to treat and prevent relapses, but the best frequency and duration of treatment remain to be defined. Pediatrics 2014;134: e1206–e1210
Giant cell hepatitis with autoimmune hemolytic anemia (GCH-AHA) is a rare disease affecting infants and young children, characterized by progressive liver disease with giant cell transformation associated with Coombs-positive hemolytic anemia.1,2 Although autoantibodies are typically negative, Coombs positivity and the response to immunosuppressive treatment suggest that GCH-AHA is an autoimmune disease. Nevertheless, it is more difficult to control than autoimmune hepatitis (especially at onset), with only 70% of long-term survival with the native liver despite aggressive immunosuppression.2 Treatment strategies include steroids, azathioprine, mycophenolate mofetil (MMF), calcineurin inhibitors, sirolimus, intravenous immunoglobulin (IVIg), cyclophosphamide, and splenectomy.2,6 Empirical use of rituximab has been reported to treat GCH-AHA, with diverse results.2,3,5,6 Coombs-positive AHA is well known to result from the activation of complement cascade on the red cell surface secondary to immunoglobulin G (IgG) binding.7 We recently showed that hepatocyte injury in GCH-AHA is complement mediated and that the portal inflammatory infiltrate is constituted mainly of macrophages and neutrophils, both suggesting B cell–mediated autoimmunity as the predominant mechanism of disease.8 We describe here the detailed clinical evolution of 4 children with GCH-AHA whose disease responded well to lymphocyte B depletion with rituximab.

PATIENT DESCRIPTION

Patient characteristics at diagnosis are summarized in Table 1. Three of the 4 children were girls. In 2 patients the onset of AHA preceded that of liver disease by 3 to 4 months. Diagnosis of GCH-AHA was rapidly made once liver signs and symptoms appeared. Liver disease manifested as elevation of liver enzymes up to 134 times the upper limit of normal at 3 to 16 months of age. Three out of 4 patients had jaundice at presentation. They all had Coombs-positive hemolytic anemia and negative autoantibodies. All other known causes of toxic, viral, or metabolic liver disease were excluded. Liver biopsy showed giant cell transformation of hepatocytes and mild or moderate fibrosis. Three patients had moderate portal and periportal inflammation, with portal infiltrate constituted predominantly of neutrophils, eosinophils, and lymphocytes, with rare plasma cells (PCs; Fig 1). Patient 4 showed portal inflammation only later in the course of the disease. All patients had C5b-9-positive hepatocytes (Fig 1), with few CD3-, CD20- or CD68-positive cells.

All patients were initially treated with high-dose oral or intravenous corticosteroids (2 mg/kg per day). Patients who had previously been diagnosed with AHA (patients 1 and 3) were already on steroids at GCH presentation. Azathioprine was rapidly added for patients 1 to 3 (0–2 months after diagnosis) and titrated according to 6-thioguanine levels. Response to standard therapy was poor in all patients (Fig 2). They all received IVIg starting from 0 to 3 months since diagnosis. In patients 1 and 2, IVIg administration was stopped after 3 and 1 doses because of lack of response and allergic reaction, respectively. Some improvement was noted in patient 4 after 2 doses. In patient 3 alanine aminotransferase (ALT) levels improved slightly upon IVIg, and she received a total of 42 doses. Nevertheless, all 4 patients had to start a stronger immunosuppressant (cyclosporine was added for patients 1–3 and sirolimus for patient 4) 3 to 5 months after onset and were treated for 20 to 51 months. Patient 3 had such a severe disease that she had to be switched to MMF and tacrolimus while maintaining steroids. Furthermore, she developed an autoimmune thrombocytopenia, which responded only partially to immunosuppression. Despite aggressive treatment, the disease was difficult to control in all patients, and each remission was followed by a relapse upon lowering of immunosuppression. GCH activity (as measured by ALT levels) was not correlated with AHA activity (measured by hemoglobin levels; N=168, including 3 patients with GCH-AHA).

<table>
<thead>
<tr>
<th>TABLE 1 Clinical, Biochemical, and Histologic Features at Diagnosis of the 4 Children With GCH-AHA</th>
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<tbody>
<tr>
<td><strong>Patient</strong></td>
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<tr>
<td>---------------</td>
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<tr>
<td>1</td>
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<td>2</td>
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**Liver histology**

<table>
<thead>
<tr>
<th><strong>Patient</strong></th>
<th><strong>Giant cell transformation</strong></th>
<th><strong>Portal and periportal inflammation</strong></th>
<th><strong>Portal infiltrate</strong></th>
<th><strong>Parenchymal microabscesses</strong></th>
<th><strong>Portal fibrosis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+++</td>
<td>++</td>
<td>Neutrophils +++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>+++</td>
<td>+</td>
<td>Neutrophils +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+++</td>
<td>+</td>
<td>Eosinophils ++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>Lymphocytes ++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Neutrophils**

**Eosinophils**

**Lymphocytes**

**Parenchymal microabscesses**

**Portal fibrosis**

**ULN,** upper limit of the normal.
$R^2 = 0.09, P < .0001)$. No correlation was found between flares of hepatitis or anemia and IgG levels ($N = 64, R^2 = 0.005, P = ns$). Patient 3 was the only one to show an elevation of $\gamma$-glutamyl transferase (GGT) levels at each relapse. Nevertheless, such a hyper-GGT was not correlated with ALT levels ($N = 109, R^2 = 0.035, P = ns$). Autoantibodies were periodically checked in all patients and were always negative.

Rituximab was administered at the standard dose (375 mg/m$^2$), once weekly for 3 to 5 consecutive weeks. Although such a cycle induced remission in all subjects, patients 2, 3, and 4 relapsed 12 to 17 months after the last injection. Patient 2 received a total of 10 doses of rituximab, whereas patient 4 received a second 4-dose cycle followed by another dose after 6 months. Patient 3 received 2 cycles of 3 weekly injections at 12-month interval. The relapse after the second cycle was milder and allowed the interval between injections to be increased. Whereas AHA was subsequently in remission, and ALT levels never flared above 2 to 3 times the upper limit of the normal, a total of 17 doses was needed for liver disease to be completely controlled. Steroids could be stopped only after several doses of rituximab, while MMF was continued. The extremely high cumulative dose of steroids, spanning over $>13$ years, resulted in osteoporosis, necessitating bisphosphonates, and major growth impairment ($-3.1$ SDs). However, patients 1, 2, and 4, who were treated with rituximab earlier in their disease course, received a much lower dose of corticosteroids and presented with no long-term consequences. No side effects to rituximab were registered for any of the patients. No severe infections were noted except for an episode of seizures (secondary to possible viral encephalitis for which no known virus could be detected) in patient 2 $>1$ year after the last injection of rituximab.

At last follow-up visit, all patients were alive with their native liver 2 to 16 years after disease presentation. Maintenance treatments were azathioprine and cyclosporine for patient 1, cyclosporine alone for patient 2, MMF for patient 3, and sirolimus for patient 4. They were asymptomatic except for a mild hypertrichosis in patient 1, had normal ALT levels, and presented no clinical or biological signs of chronic liver disease.

**DISCUSSION**

First described by Bernard et al in 1981, GCH-AHA is a rare and extremely severe autoimmune disease of infancy
and early childhood that often leads to liver failure.\textsuperscript{2} Response to standard immunosuppressive therapy is inadequate in most patients, and liver transplantation is complicated by a high rate of recurrence.\textsuperscript{2,10–12} The 4 cases presented here share a severe course of the disease with an incomplete response to both standard and aggressive immunosuppression.

We recently showed that GCH-AHA is probably caused by a humoral immune mechanism.\textsuperscript{8} Rituximab is a chimeric anti-CD20 monoclonal antibody specifically targeting B lymphocytes. We described its efficacy in treating refractory autoimmune hepatitis.\textsuperscript{13} Its use in treating GCH-AHA has been empirically tried in 5 children.\textsuperscript{2,3,5,6} In 2 of these patients, the effect of rituximab was shown to be temporary.\textsuperscript{2,5} Except for patient 1, all patients presented here showed several relapses of both anemia and the liver condition over the years after the first series of injections. Because stem cells and early pro-B cells are CD20-negative, they are not affected by rituximab. Furthermore, rituximab has no effect on antigen-presenting cells or autoreactive CD4+ T cells. Restoration of B cells in the peripheral blood occurs within 5 to 13 months.\textsuperscript{14,15} Relapses are possible because of repletion of the B cell pool. After repletion, B cells can be activated upon presentation of a yet-undefined autoantigen through antigen-presenting cells or the interaction with autoreactive T cells. Furthermore, depletion of B lymphocytes is not complete, and survival of memory B cells is possible, as is persistence of long-lived PCs.\textsuperscript{15} Infections could trigger such relapses through a cross-reactive antigen. Relapses after liver transplant are probably secondary to the lack of action of calcineurin inhibitors on humoral immunity.\textsuperscript{10,11} A role of elevated serum B lymphocyte stimulator levels in inducing relapses has been hypothesized for rheumatoid arthritis and would be interesting to study in GCH-AHA.\textsuperscript{16} Although the pathogenesis of relapses is not clear, the need for repeated injections of anti-CD20 to treat (and perhaps to prevent) relapses is clinically evident. The frequency and

**FIGURE 2**

Response to therapy for patients 1–3 (A–C, respectively): ALT (black line), GGT (light gray line), and hemoglobin (dark gray line) levels over the time. Administered treatments are shown below each graph. The dose of steroids is shown by the thickness of the gray line (the highest level representing 2 mg/kg per day). Rituximab doses are represented by arrows.
overall duration of such “maintenance” treatment are unknown. Rovelli et al. described a patient who failed to respond to rituximab and was subsequently treated with alemtuzumab (an anti-CD52 monoclonal antibody targeting all circulating lymphocytes, monocytes, and dendritic cells). PCs are usually CD20-negative effector B lymphocytes responsible for antibody production. Nevertheless, autoreactive PCs were shown to be CD20-positive and different from protective antimicrobial PCs targeting all circulating lymphocytes, (an anti-CD52 monoclonal antibody subsequently treated with alemtuzumab failed to respond to rituximab and was attributable to the antibodies PCs. The lack of immediate effect could be attributable to the antibodies’ half-life (20 days) rather than to surviving PCs. For this reason, concurrent short-term treatment with steroids is recommended, whereas the use or association of alemtuzumab might not be justified.

Side effects of corticosteroids are well known. We show here that an early use of rituximab has an important steroid-sparing effect, reducing long-term side effects on bone mineralization and growth.

Furthermore, rituximab as first-line treatment could reduce the need for aggressive immunosuppression and the potentially severe side effects associated with calcineurin inhibitors, cyclophosphamide, or splenectomy. Based on the data available so far, GCH-AHA appears to be mediated by autoantibodies. The lack of association between disease activity and IgG plasma level, together with liver biopsy findings and Coombs positivity, strengthen the humoral hypothesis. Overall, we show here that there is a strong rationale for treating GCH-AHA with rituximab. Early treatment could reduce the use of corticosteroids and prevent side effects. Nevertheless, steroids should be associated with rituximab for a few weeks, to account for antibodies’ half-life. Repeated injections over time are needed to treat and prevent relapses, but the best frequency and duration of treatment are still to be defined.

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