with defects of the branchial arches such as in FAVS. Hence, all babies of FAVS should have an estimation of serum calcium and phosphorus as a screening test, so that physical and mental retardation could be prevented.

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Rituximab for Treatment of Autoimmune Hemolytic Anemia

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We report the successful use of rituximab as single treatment modality in a five-month-old boy with fulminant warm autoantibody autoimmune hemolytic anemia, resistant to standard treatment. On admission, laboratory tests showed a profound anemia with a hemoglobin of 2.6 g/dL. Indirect and direct antiglobulin tests were strongly positive, and nonspecific IgG autoantibodies were detected. Two days of intravenous corticosteroids (methylprednisolone 4mg/kg) and immunoglobulins (1g/kg) did not halt the hemolysis and the infant was severely transfusion-dependent. Rituximab 375mg/sq m weekly was given for 4 weeks, the hepatosplenomegaly gradually regressed, the lymphocytes normalized and he is free from hemolysis two years after treatment.

Key words: *Autoimmune hemolytic anemia, Rituximab*.

Autoimmune hemolytic anemia (AIHA) constitutes a group of diseases classified on the basis of the temperatures at which the autoantibodies exhibit their maximal reactivity to erythrocytes; warm AIHA has maximal reactivity at 37°C, and cold AIHA at 28-31°C [1]. Glucocorticoids and/or intravenous immunoglobulins are the mainstay of treatment in the majority of patients with warm AIHA [2,3]; however, when these treatments fail patients often require cytotoxic drugs or splenectomy.

We describe a 5-month old boy with a fulminant type warm AIHA resistant to the standard treatment who was successfully treated with rituximab.

CASE REPORT

A 5-month-old boy with an uneventful prior medical history was admitted to a regional hospital for investigation following two days of sudden onset pallor, malaise and anorexia. At presentation, his laboratory tests revealed a profound anemia with a hemoglobin (Hb) of 2.6 g/dL, RBC 0.9×10^9/L, MCV 98.2 fl, RTC 5.7%, WBC 15.4×10^9/L and platelets 638×10^9/L; total bilirubin levels were 127 micromol/L, with a direct fraction of 19.2 micromol/L. Indirect and direct antiglobulin tests were strongly positive, and nonspecific IgG autoantibodies were detected. Serum immunoglobulin levels were within the normal range for his age. Chest radiography was normal, and abdominal ultrasound revealed a mild splenomegaly and hepatomegaly. He received intravenous immnunoglobulins and corticosteroids; however the hemolysis continued and a transfusion of packed red cells was followed by severe hemoglobinuria. He then

underwent a partial exchange transfusion with 450 mL of packed red cells to reduce the circulating autoantibody levels, and was referred to our Institution.

On admission, he was in a generally good condition, with biochemical signs of hemolysis (LDH 1511 U/L, total bilirubin 156 microml/L, with a direct fraction of 24.2 microml/L); Hb 126g/l, RBC 3.6×10^9/L, RTC 7.2% and hepatosplenomegaly. He was given further intravenous corticosteroids (methylprednisolone 4mg/kg) and immunoglobulins 1g/kg for two days, however these again failed to halt the hemolysis and the child was severely transfusion dependent; receiving two to three transfusions of packed red cells per day. His general condition significantly deteriorated (***Fig.* 1**) with increasing hepatosplenomegaly and rising bilirubin levels (total bilirubin 764.7 micromol/L, with an increasing direct fraction of 637.9 micromol/L) and an LDH of 2780 U/L.

Eight days following admission, he was started on rituximab 375mg/sqm weekly. After the second dose his transfusion requirements and reticulocyte counts gradually reduced. The last packed red cell transfusion was given two days after the third dose of rituximab. The infant was given a total of four doses of rituximab weekly, followed by monthly infusions of immunoglobulins for the next six months and the hepatosplenomegaly gradually regressed.

At 1 year follow-up, he was physically well without any observed side-effects from the rituximab treatment. Investigations revealed WBC of 10.9×10^9/L, with normal lymphocyte counts for age, including absolute lymphocyte counts (Ly 4690 cells/µl) and lymphocyte subsets analyzed through flow cytometry on gated cells by direct immunophenotyping (CD 45+cells 40%, CD19+/CD45+ 27%, and CD20+/CD45+ 26%) at Becton Dickinson, San Jose, USA. Immunoglobulin levels were also normal. He remains well and free from hemolysis two years after treatment.

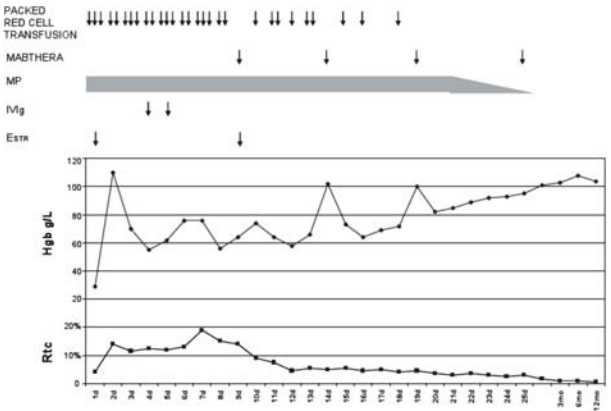

FIG. 1 *Hemoglobin and reticulocyte levels before and after the application of rituximab.*

DISCUSSION

Rituximab, intravenous immunoglobulins, immunosuppressive drugs and danazol have been shown to be effective in refractory AIHA and in poor surgical candidates [1]. Rituximab is a humanized monoclonal antibody IgG1/k directed against the CD20 antigen. Although originally created for the treatment of non Hodgkin's lymphoma, the resultant depletion in normal B lymphocytes (which are crucial for inducing and maintaining autoimmunity), has been found to improve and/or cure a wide variety of autoimmune disorders [4, 5].

The mechanism behind this immunomodulatory effect is not fully understood; as plasmocytes carry no CD20 expression on their surface, and the direct destruction of antibody secreting cells is not a plausible explanation. Furthermore, the autoantibody titer do not correlate with clinical and laboratory improvement seen following rituximab treatment. A possible mechanism of action lies with the fact that B cells are antigen presenting cells, and as such have a crucial role in activating and maintaining the response of autoreactive T cells. The "immune decay" hypothesis postulates that antibody-coated B cells are recognized by monocytes and macrophages, which thus divert them away from interacting with the autoimmune antibody complexes.

Our patient required immediate treatment with packed red cell transfusions, immunoglobulins and corticosteroids due to having an extremely low hemoglobin level, and thus investigations for an underlying cause had to be postponed. However, he had been a healthy child for the first 5 months of his life, with a positive Coombs test, and had a good response to immunosuppressive treatment with rituximab, thus congenital hemolytic anemia could be excluded. Primary immunodeficiency was also excluded with the presence of normal lymphocyte subsets and immunoglobulin levels, and the regression of his hepatosplenomegaly during follow-up.

Our case demonstrated that as a single agent rituximab was effective and safe in the context of a life-threatening and fulminant warm antibody AIHA that had been resistant to glucocorticoids. The efficacy of rituximab has also previously been established in the

treatment of children with idiopathic AIHA, following organ transplant and during the course of primary immunodeficiency [1, 3-6]. However, data on its use during the first year of life is limited to information from small case series and case reports [3,4,7]. One series described various treatment durations with patients receiving between 4 and 35 courses of rituximab [7]. Their patient whose treatment was initiated earliest (11 days following diagnosis), required fewest rituximab courses to achieve remission [7].

Our patient also achieved remission after 4 doses of rituximab which was initiated 11 days after diagnosis, thus we believe that an earlier introduction of treatment in case of refractory AIHA in children offers the possibility of complete remission with fewer cycles of rituximab. Our experience also supports the belief that through the use of rituximab, the more aggressive modes of treatment, such as splenectomy and the use of cytotoxic drugs, could be avoided.

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