Brief report

Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated, pure red cell aplasia and hemolytic anemia

Marco Zecca, Piero De Stefano, Bruno Nobili, and Franco Locatelli

Immune-mediated, acquired pure red cell aplasia (PRCA) is a rare disorder frequently associated with other autoimmune phenomena. Conventional immunosuppressive treatment is often unsatisfactory. Rituximab is a monoclonal antibody against the CD20 antigen, highly effective for in vivo B-cell depletion. An 18-month-old girl with both severe PRCA and autoimmune hemolytic anemia, refractory to immunosuppressive treatment, received 2 doses of rituximab, 375 mg/m² per week. The drug was well tolerated. After anti-CD20 therapy, substitution treatment with intravenous immunoglobulin was started. The treatment resulted in marked depletion of B cells; a striking rise in reticulocyte count ensued, with increasing hemoglobin levels, finally leading to transfusion independence. The child is now 5 months off-therapy, with normal hemoglobin and reticulocyte levels. This case suggests a role of anti-CD20 monoclonal antibody for treatment of patients with antibody-mediated hematologic disorders. (Blood. 2001;97:3995-3997)

© 2001 by The American Society of Hematology

Introduction

Acquired pure red cell aplasia (PRCA) is rare in infancy, median age of onset being usually between 50 and 60 years.1-3 The pathogenesis of the disease is immune-mediated in most cases, soluble serum inhibitors or inhibitory T cells, able to impair in vitro growth of erythroid progenitors (burst-forming unit [BFU-E], and colony-forming unit [CFU-E]), having been demonstrated in several series of patients.1-3 PRCA is frequently associated with other autoimmune phenomena, that is, production of autoantibodies directed against erythrocytes, acetylcholinesterase, smooth muscle, and so on.2 PRCA associated with autoimmune hemolytic anemia (AIHA) has been previously reported.5

Treatment of PRCA usually employs steroids and/or other immunosuppressive drugs (ie, cyclosporine-A and cyclophosphamide), or immunomodulating agents such as immunoglobulin, with response rates to these therapies ranging from 30% to 55%.2,3

Rituximab is a humanized, murine, monoclonal antibody (MoAb) directed against the CD20 antigen, expressed on pre-B lymphocytes and on mature B lymphocytes.5,6 Rituximab has been demonstrated to be highly effective for in vivo B-cell depletion, B lymphocytes becoming undetectable in peripheral blood after a single infusion, and recovering only 6-9 months after discontinuation of treatment.5,6 The antibody has been recently introduced for treatment of B-cell lymphomas.7-10

We describe the case of an 18-month-old child with immune-mediated PRCA and AIHA, refractory to first- and second-line immunosuppressive therapy, who was successfully treated with anti-CD20 MoAb.

Study design

An 18-month-old Caucasian girl was hospitalized due to recent onset of pallor and listlessness. Physical examination at admission was unremarkable, with the exception of pallor and mild jaundice; the patient did not present either spleen or liver enlargement. Full blood count at admission showed normochromic-normocytic anemia (hemoglobin = 5.6 g/dL) with reticulocytopenia (absolute reticulocyte count = 7 x 10⁹/L) and normal white blood cell and platelet counts. Total and unconjugated bilirubin levels were increased (2.31 mg/dL and 1.77 mg/dL, respectively), and serum haptoglobin was undetectable. Tests for hereditary hemolytic anemias, as well as direct and indirect Coombs tests, were negative. Evaluation of the blood film revealed the presence of spherocytes, whereas erythrocyte fragments were not detectable. Serologic tests for parvovirus B19, human cytomegalovirus, and Epstein-Barr virus were negative. Chest x-ray was normal. A blood count performed 6 months earlier had shown normal hemoglobin (12 g/dL).

A bone marrow aspirate performed at admission demonstrated normal representation of myeloid and megakaryocyte precursors, but nearly absent erythroid precursors. In vitro cultures of patient light-density bone marrow cells, performed in triplicate as described previously,11 demonstrated that, compared with 3 healthy bone marrow donors, a significant reduction of colonies from erythroid progenitors was present, with patient and controls BFU-E and CFU-E being 20 ± 4 versus 88 ± 11 and 32 ± 3 versus 67 ± 5, respectively. By contrast, growth of the patient’s granulocyte-macrophage progenitors (CFU-GM) was normal. The patient’s plasma profoundly inhibited growth of BFU-E from healthy controls (93 ± 16 and 24 ± 6 without and with patient’s plasma), suggesting the existence of soluble serum factors responsible for both PRCA and hemolysis.

Due to a steadily decreasing hemoglobin level, the child was transfused. Repeated administration of red blood cell concentrates was required in order to maintain hemoglobin above 6 g/dL (Figure 1). In the following weeks, she remained transfusion-dependent, with erythrocyte consumption much higher than that compatible with hypoproliferative anemia (transfusion of 15-20 mL/kg of packed red blood cells every 5-7 days was necessary). In detail, during a period of 10 weeks, 13 erythrocyte transfusions were administered. Serum bilirubin levels remained above normal levels, whereas serum haptoglobin was constantly undetectable. Despite clinical and biochemical signs of continuing hemolysis, the direct antiglobulin test (DAGT), repeatedly performed during this time, remained

© 2001 by The American Society of Hematology
Reticulocyte count and in reducing erythrocyte consumption. Concomitant
treatment with cyclosporine-A during 6 weeks was equally ineffective
compared to placebo. Arrows show the 2 infusions of

Rituximab was administered intravenously at the dose of 375 mg/m² as a
4-hour infusion, once weekly for a total of 2 doses. Before each infusion,
the child received pre-medication with methylprednisolone and diphenhy-
dramine. The drug was well tolerated and the child did not present any
adverse reaction or side effects. Substitutive treatment with intravenous
immunoglobulin (400 mg/kg every 3 weeks) was given, in order to prevent
treatment-induced hypogammaglobulinemia.

Results and discussion

Treatment with rituximab resulted in marked depletion of B cells in
peripheral blood, as demonstrated by the percentage of CD19⁺ and
CD20⁺ cells dropping from the pretreatment values of 12% and
11%, respectively, to values less than 0.5% for both antigens 2 days
after the first infusion.

Shortly after the first anti-CD20 MoAb infusion, the patient
showed a striking rise in reticulocyte count, with a maximum value of
347 × 10⁹/L, achieved 2 weeks after starting the treatment. Progressive increase of
hemoglobin level and achievement of
transfusion independence (Figure 1) followed the rise in reticulo-
cytes. Bilirubin and haptoglobin levels normalized, as well. The
course of the response was as expected and gave further support to
a diagnosis of AIHA with concomitant antibody-mediated PRCA.

The child, 5 months after MoAb therapy, is transfusion-
independent, with normal hemoglobin and reticulocyte levels. She
is no longer receiving immunosuppressive treatment. The percentage
of the patient’s B lymphocytes is still below 1%. During the
observation period, the child did not develop any significant
infectious complication.

As mentioned, so far only one case of immune-mediated thrombocy-
topenia, successfully treated with anti-CD20 MoAb, has been exten-
sively reported in the literature. In that patient, the administration of 4
doses of rituximab induced complete and long-lasting normalization of
platelet count. Treatment of patients with AIHA using rituximab has
been preliminarily reported with variable response.

In our experience, response to anti-CD20 infusion occurred 4
days after the first administration, and it was sustained, allowing
discontinuation of all other immunosuppressive therapies. The rapid response and disappearance of B cells from peripheral blood
suggested that 2 doses of monoclonal antibody might be sufficient,
thus saving costs.

Recovery of B lymphocytes after treatment with rituximab has
been described to start from 6 to 9 months after the last administra-
tion; a normal B-lymphocyte number is usually achieved only 12
months after treatment discontinuation. As a consequence, a
significant reduction in immunoglobulin M (IgM) and IgG serum
levels is to be expected, with a possible risk of infectious complications, as recently reported.

For this reason, we gave replacement therapy with intravenous immunoglobulins.

The outcome of childhood severe, immune-mediated, hematologic
cytopenia with conventional immunosuppressive approaches is
sometimes unsatisfactory. For this reason, the recent introduc-
tion of new immunomodulating agents, such as MoAbs approved
for in vivo use, represents an appealing and promising treatment
modality for patients with the most severe and/or refractory forms
of disease. In refractory or chronic disease, use of rituximab is
attractive also because it could reduce or avoid some side effects of
prolonged therapy with steroids (eg, fluid retention, severe cata-
bolic damage, hyperglicemia, avascular necrosis of bone, growth
impairment) and/or other aspecific immunosuppressive drugs.

In conclusion, even though a longer follow-up is required to
assess the long-term efficacy of this treatment, this case provides a
basis for future prospective trials aimed at defining the role of
anti-CD20 MoAb for antibody-mediated hematologic disorders.

Acknowledgments

We would like to thank Rita Maccario for the immune-phenotype
study of peripheral blood lymphocytes and Patrizia Comoli for help in revising the manuscript.

References

1. Freedman MH. Pure red cell aplasia in childhood
and adolescence: pathogenesis and approaches
2. Alter BP, Young NS. Bone marrow failure syn-
dromes. In Nathan DG, Oski FA, eds. Hematol-
ysis of infancy and childhood. Philadelphia, PA:
WB Saunders; 1993:216-316.
3. Erslev AJ. Pure red cell aplasia. In Beutler E,
Lichtman MA, COLLER BS, KIPPS TJ, eds. Williams
448-450.
4. Hegde UM, Gordon-Smith EC, Worledge SM.
Reticulocytopenia and “absence” of red cell auto-
antibodies in immune haemolytic anaemia. Br
Czuczman MS, Williams ME. Rituximab chimeric
anti-CD20 monoclonal antibody therapy for re-
lapsed indolent lymphoma: half of patients re-
spond to a four dose treatment program. J Clin
of B-cells in vivo by a chimeric mouse monoclonal
7. Coiffier B, Haioun C, Ketterer N, et al. Rituximab (anti-CD20 monoclonal antibody) for the treat-
ment of patients with relapsing or refractory ag-
gressive lymphoma: a multicenter phase II study. 
8. Leget GA, Czuczman MS. Use of rituximab, the 
new FDA-approved antibody. Curr Opin Oncol. 
IDEC-C2B8 (rituximab) anti-CD20 antibody 
therapy in patients with relapsed low-grade non-
2188-2195.
imab therapy in hematologic malignancy patients 
with circulating blood tumor cells: association with 
increased infusion-related side effects and rapid 
blood tumor clearance. J Clin Oncol. 1999;17: 
791-795.
11. CarloStella C, Ganser A, Hoelzer D. Detective “in 
vitro” growth of hematopoietic progenitor cells CFU-
GEMM, CFU-Mk, BFU-E and CFU-GM in the ac-
quired immunodeficiency syndrome (AIDS). 
Anti-CD20 chimeric monoclonal antibody treat-
ment of refractory immune-mediated thrombocy-
topenia in a patient with chronic graft-versus-host 
13. Lee E, Zamkoff KW, Gentile TC, Zimrin A. Rituxan 
in the treatment of autoimmune hemolytic anemia 
14. Grossi A, Santini V, Longo G, Balestri F, Rossi-
Ferrini P. Treatment with anti-CD20 antibodies of 
patients with autoimmune thrombocytopenia with 
or without hemolytic anemia; worsening in the 
hemoglobin level. Blood. 2000;96(suppl 1): 
253a.
15. Berenstein S, Tjonnfjord GE, Gjertsen BT et al. 
Rituxan (Rituximab) therapy for chronic cold ag-
South San Francisco: Genentech Bio-Oncology 
and ADEC Pharmaceuticals Corporation; 1998.
17. Dervite I, Hober D, Morel P. Acute hepatitis B in a 
patient with antibodies to hepatitis B surface anti-
gen who was receiving Rituximab. N Engl J Med. 
18. Sharma VR, Fleming DR, Stone SP. Pure red cell 
aplasia due to parvovirus B19 in a patient treated 
Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated, pure red cell aplasia and hemolytic anemia

Marco Zecca, Piero De Stefano, Bruno Nobili and Franco Locatelli