Aplastic anemia successfully treated with rituximab: The possible role of aplastic anemia-associated autoantibodies as a marker for response

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Title: Aplastic anemia successfully treated with rituximab: The possible role of aplastic anemia-associated autoantibodies as a marker for response

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Abstract

A 1-year-old Japanese male developed hepatitis-associated aplastic anemia (AA) and anti-thymocyte globulin (ATG) plus cyclosporine A (CsA) was administered without any appreciable effects. Laboratory examination of the patient's serum obtained before therapy revealed various autoantibodies, such as PA-IgG, anti-platelets, anti-single-stranded DNA (ssDNA), and anti-double-stranded DNA (dsDNA) antibodies (Abs) in addition to anti-DRS-1 Abs and anti-moesin Abs, both of which are known to be detectable in approximately 40% of all patients presenting with AA. He was therefore treated with 17.5 mg/kg/d rituximab 5.5 months after ATG/CsA therapy. The same rituximab therapy was repeated 3 times once a month thereafter. His neutrophil counts started to increase 50 days after the first rituximab therapy and he achieved a complete remission at 16 months after the last rituximab administration. All of the autoantibodies including anti-ssDNA, dsDNA, DRS-1 and moesin Abs became undetectable when he attained the remission. Anti-CD20

monoclonal antibody therapy may be effective in a subset of patients with AA characterized by the presence of autoantibodies.

Key words: Aplastic anemia; Rituximab; autoantibody

Rituximab, a chimeric human/mouse monoclonal antibody targeting the CD20 antigen, has been successfully used for treatments of various autoimmune hematologic diseases such as immune thrombocytopenic purpura (ITP), autoimmune hemolytic anemia (AIHA) and thrombotic thrombocytopenic purpura (TTP) (1-6). In this case report, we describe a child with aplastic anemia (AA) who failed to respond to a first course of anti-thymocyte globulin (ATG) plus cyclosporine A (CsA) therapy but later improved with rituximab treatment. To our knowledge, this is the first pediatric AA case which was successfully treated with rituximab.

Method

Detection of anti-DRS-1 and anti-moesin autoantibodies by Western blotting analysis

Approximately 5 μg of recombinant native DRS-1 or moesin protein per lane were electrophoresed in a 12% polyacrylamide gel and transferred to a PVDF membrane. The membrane was incubated in 3% BSA-PBS containing serum diluted 1:200 from this AA patient or a healthy individual (7, 8).

Case report

A 15-month-old Japanese male was admitted to a nearby hospital due to a high fever and intraoral bleeding in February of 2006. He was born to healthy nonconsanguineous parents and was normally nourished. The family history did not include hematologic or malignant diseases. Physical examination on admission showed petechiae and no splenomegaly, and

the liver was palpable 2 cm below the right costal arch. His complete blood count (CBC) showed a red blood cell (RBC) count of 3.71 x 10^{12}/L and hemoglobin (Hb) level of 11.0 g/dL. A white blood cell (WBC) count of 21.3 x 10^{9} /L with a differential count of eosinophils 0%, basophils 0%, neutrophils 50%, lymphocytes 47%, and monocytes 3%, and a platelet count of 9.0 x 10^{9}/L. Bone marrow (BM) aspiration and biopsy showed a slight hypoplasia with relatively reduced granulocytes. No signs of myelodysplasia or an increase in the number of atypical cells were observed. The G-banding of 20 metaphase BM cells showed all cells to be a normal karyotype, 46, XY. No Abs specific to viruses were found, including the hepatitis A virus, hepatitis B virus, hepatitis C virus, Epstein-Barr virus or parvovirus B19 other than anti-cytomegalovirus IgG Abs. Autoantibody screening revealed the presence of Abs against smooth muscle, cardiolipin, single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), SS-A, SS-B, Sm, platelets, and PA-IgG. Anti-liver/kidney microsome type 1 Abs were of a borderline titer, but all of antinuclear Abs, rheumatoid factor

and anti-neutrophil Abs were negative. The haptoglobin level was within the normal range. Blood chemistry revealed abnormal values as follows: IgG 2035 mg/dL (normal range 820–1740 mg/dL), AST 203 IU/L (normal range 10–40 IU/L), ALT 609 IU/L (normal range 5–40 IU/L), LDH 304 IU/L (normal range 115–210 IU/L), ALP 838 IU/L (normal range 104–338 IU/L) and T-bil 1.3 mg/dL (normal range 0.2–1.0 mg/dL). A contrast-enhanced CT study of the patient's abdomen was normal. He was diagnosed to have autoimmune hepatitis (AIH) with ITP. Bolus methylprednisolone (250 mg/d for 3 days) followed by prednisolone (2 mg/kg/d) and high-dose IVIG (0.4 g/kg/day) for 5 days were administered from March 18th, 2006. His AIH resolved in 4 weeks but pancytopenia further progressed, necessitating frequent RBC and platelet transfusion. On June 29th 2006, a CBC showed a RBC count of 2.47 x 10^{12}/L and Hb 7.5 g/dL, and a reticulocyte count of 8.0 x 10^{9}/L, a WBC count of 2.3 x 10^{9} /L with eosinophils 1%, basophils 0%, neutrophils 6%, lymphocytes 92%, and monocytes 1%, a neutrophil count of 0.1 x 10^{9}/L and a platelet count of 58 x 10^{9}/L. BM

aspiration and biopsy showed a severe hypocellularity with fatty change. The G-banding of 7 metaphase BM cells showed all cells to have a normal karyotype, 46, XY. Very severe AA was diagnosed (Fig. 1A). Abs specific to DRS-1 and moesin, which are detectable in approximately 40% of patients with AA (7, 8), were positive in the serum of this patient. No increase in the glycophosphatidylinositol-anchored protein–deficient erythrocytes and granulocytes was observed. On July 5th 2006, ATG (Lymphoglobuline; Aventis Behring, King of Prussia, PA; 15 mg/[kg/day], 5 days) plus cyclosporine (CsA; Novartis, Basel, Switzerland; 4-12 mg/[kg/day]) was administered according to the standard protocol (9), but no increases in blood cells count were observed 5.5 months later (Fig. 1B). HLA-identical donors were unavailable from the Japan Marrow Donor Program either. Because the patient was positive for various autoantibodies, the effect of anti-B cell therapy was anticipated. Therefore, 17.5 mg/kg/d of rituximab was started from December 19th of 2006, 5.5 months after ATG therapy. A CBC showed a RBC count of 2.33 x 10^{12}/L and Hb 6.5 g/dL, and a

reticulocyte count of 4.0 x 10^9/L, a WBC count of 2.0 x 10^9 /L, a neutrophil count of 0.2 x 10^9 /L and a platelet count of 53 x 10^9/L at the time of the first rituximab administration. Written informed consent was obtained from his parents prior to the treatment. The same dose of rituximab was given monthly 4 times, and 80 mg/d CsA was continued. The neutrophil count of the patient started to rise 50 days after the first rituximab administration and then continued to rise thereafter. Twenty months after the first rituximab therapy his neutrophil was more than 5.0 x 10^9/L, the Hb level 11 g/dL, and the platelet count 158 x 10^9/L (Fig. 2). A BM biopsy performed at 10 months of the first rituximab therapy showed myeloid and erythroid hyperplasia with reduced megakaryopoiesis (Fig. 1C), and another BM biopsy performed at 22 months of the therapy revealed a hypercellularity of all three lineage of cells (Fig. 1D). Abs against ssDNA, dsDNA, SS-A, SS-B, Sm, DRS-1 and moesin became undetectable in August of 2008 (Fig. 2), and the titers of PA-IgG and anti-cardiolipin Abs were reduced from 142.9 to 71.1 ng/10^7cells and from 39.1 to 17.9 U/mL, respectively.

From January to March in 2008, the patient had fever and inflammatory bowel disease of unknown etiology. Severe thrombocytopenia recurred after high-dose IVIG (0.3 g/kg/day) was administered to treat the bowel disease. A BM biopsy revealed abundant megakaryocytes suggestive of thrombocytopenia due to increased platelet destruction secondary to IVIG therapy (Fig. 2). Four years after the rituximab therapy, his CBC was normal, and his AA was in complete remission (CR) as of October of 2010.

Discussion

Immune-mediated suppression or destruction of hematopoietic stem cells is a major mechanism implicated in the pathophysiology of AA (10-12), where aberrant T-cell responses to autoantigens lead to excessive production of various cytokines, including interferon-γ (IFN- γ) and tumor necrosis factor-α (TNF- α) in the BM (13-15). Immunosuppression with ATG and/or CsA targeting autoreactive T cells has been successfully applied to resolve such

immune-mediated AA (16-18). Antibody-mediated stem cell insults may also be responsible for BM failure in some patients with AA, but only one case of adult AA which responded to rituximab has been reported (19).

Rituximab is thought to induce a remission of autoimmune cytopenias such as ITP, AIHA and TTP by eliminating circulating $CD20^+$ B cells including B cells capable of producing autoantibodies. Rituximab has also to have an indirect effect on cellular immunity (6, 20). The elimination of $CD20^+$ B-cells may indirectly normalize abnormalities in the cellular immunity of patients with ITP such as T-cell receptor Vβ skewing, a decrease in both the T-helper cell type 1 (Th1)/Th2 ratio and the Fas ligand expression on Th1 and Th2 cells, and also the regulatory T cell number (21, 22). Increases in regulatory T cells have been reported after rituximab treatment of patients with SLE (23, 24).

Recent studies identified several autoantibodies against kinectin (25), postmeiotic

segregation increased 1 (26), DRS-1 (7), moesin (8) and hnRNPk (27) in the sera of patients with AA. Among these autoantibodies, Abs against moesin, an intracellular protein that links the cell membrane and cytoskeleton, is unique in the way that it can enhance the secretion of TNF-α and INF- γ from PBMCs of AA patients through the activation of the ERK1/2 pathway which is provoked by direct binding to moesin on the cells (28, 29). This suggests that the elimination of such functional autoantibodies by rituximab therapy may restore hematopoiesis in AA patients by way of abolishing the excessive secretion of TNF- α and IFN- γ.

Some AA patients who failed to respond to ATG/CsA within 6 months of the therapy, conversely showed a late response and thereby eventually achieved transfusion-independence (18). It therefore cannot be denied that the CR obtained after rituximab therapy was induced by ATG/CsA. However, our patient did not show any signs of hematologic improvement, such as an increase in the platelet or reticulocytes counts at the time of the rituximab

administration, which was day 168 of the ATG therapy. His neutrophil, reticulocyte and platelet count began to increase on day 50, day 158 and day 309 of the first rituximab therapy, respectively. Given the fact that it takes several weeks until the titer of pathogenic autoantibodies sufficiently decreases after rituximab administration, it seems more reasonable to ascribe the hematologic recovery to the effect of rituximab than to the late effect of ATG. The disappearance of AA-associated autoantibodies including anti-DRS-1 Abs and anti-moesin Abs after rituximab supports the role of rituximab in the restoration of the patient's marrow failure.

In conclusion, we herein presented the first case where rituximab was successfully used as an alternative treatment for a pediatric patient with severe AA who was refractory to standard ATG/CsA therapy. The presence of autoantibodies such as anti-DRS-1 Abs and anti-moesin Abs may be useful for predicting response to rituximab therapy in AA patients. The results of this case warrant a clinical trial using rituximab for the treatment of patients

with AA refractory to ATG.

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Conflict of interest

The authors declare no conflict of interest.

Figure legends

Figure 1. Changes in the bone marrow cellularity associated with rituximab. A, before therapy; B, 2 months after ATG/CsA therapy; C, 10 months after the first rituximab therapy; D, 22 months after the first rituximab therapy.

Figure 2. Clinical course after immunosuppressive therapy. PC: platelet concentrate, PSL: prednisolone, +: positive, -: negative. The black solid line and gray solid line indicate hemoglobin and the absolute neutrophil count, respectively. The dotted line indicates the platelet count.

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Figure 1

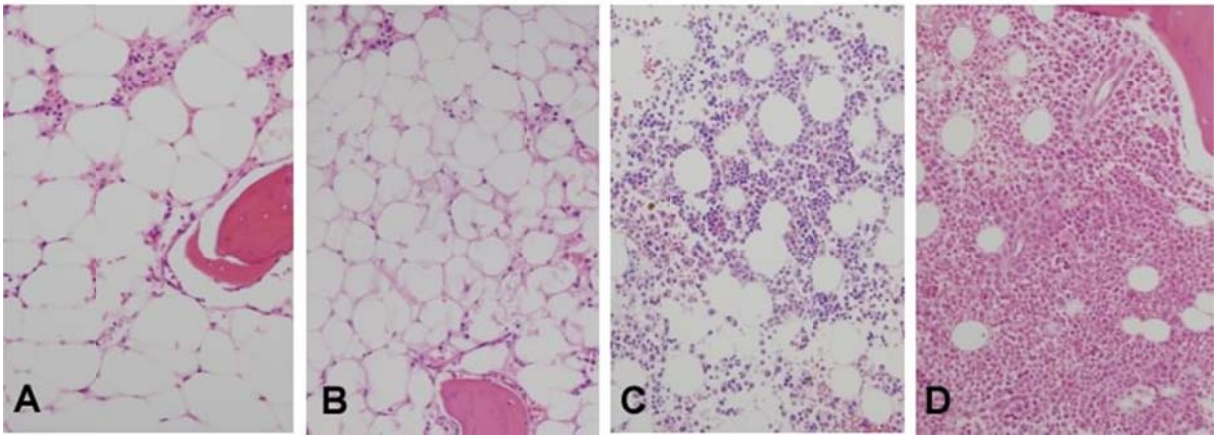

Figure 2