Rituximab for prevention of delayed hemolytic transfusion reaction in sickle cell disease

Delayed hemolytic transfusion reaction (DHTR), a life-threatening transfusion complication in sickle cell disease (SCD), is characterized by a marked hemoglobin drop with destruction of both transfused and autologous red blood cells (RBCs) and exacerbation of SCD symptoms. One mechanism of RBCs destruction is auto-antibody production secondary to transfusion. As rituximab specifically targets circulating B cells, we thought that it could be beneficial in preventing this immune-mediated transfusion complication. We report the case of a SCD patient who previously experienced DHTR with auto-antibodies and who needed a new transfusion. DHTR recurrence was successfully prevented by rituximab administration prior transfusion, supporting the safe use of rituximab to prevent DHTR in SCD patients as a second line approach when other measures failed.

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Introduction

Delayed hemolytic transfusion reaction (DHTR) is a life-threatening complication frequently reported in SCD patients.[1-4] The mechanisms of DHTR are not well understood as serological findings do not always provide a simple explanation for hemolysis, such as the presence of clinically significant allo-antibodies against red blood cells (RBCs). The hallmarks of DHTR in SCD are a dramatic drop in post-transfusion hemoglobin (Hb) caused by the destruction of both donor and recipient red blood cells (RBCs), presence of SCD related manifestations and hemolysis exacerbation by further transfusion.[5] Destruction of autologous RBCs, called bystander immune hemolysis,[6] and transfused RBCs can be triggered by auto-antibodies produced as a result of transfusion, with development of an acute auto-immune hemolytic anemia. In SCD, auto-antibodies may have a strong hemolytic capacity as RBCs are more susceptible to fix antibodies and complement, mainly because of increased phosphatidylserine exposure.[6-8] Transfusion can elicit auto-immunization against RBCs, specially when RBC allo-antibodies are produced.[9] This mechanism is also well known in post-transfusion purpura, a characteristic reaction caused by allo-antibodies against platelets.[10] However, in some cases there is no demonstrable allo-antibodies.

Rituximab, a chimeric mouse/human monoclonal antibody which binds to the transmembrane CD20 antigen, rapidly eliminates circulating B cells with a potential capacity to prevent auto-antibody production by targeting autoreactive B cells.[11] Rituximab could also be involved in other mechanisms as B lymphocytes are antigen presenting cell through their B cell receptor and can cooperate with T cells during allo-immunization.[12] Then, one may speculate that rituximab could be helpful in preventing auto-antibody production following transfusion.

We report the case of a SCD patient with a prior history of post-transfusion life threatening hemolytic anemia, mainly caused by auto-antibodies, who could be successfully transfused after being treated with rituximab.

Patient case report

The 33-year-old SCD patient was poly-immunized with anti-C, anti-RH23, anti-Fya, anti-S, anti-Ytb. He had already experienced two DHTR with both auto and allo-antibodies following transfusion for orthopedic surgery. He was scheduled a third time in 2005 for hip replacement. Because of the high risk of DHTR, he was surveyed very closely (Figure 1A). He received 7 cross-matched-compatible units at day 0 of surgery. On days 5 and 8, sera were still compatible with samples of units received at day 0. Direct antiglobulin test (DAT) and eluate were negative. Hb remained stable at 7 g/dL. On day 14, the patient presented pain, fever and features of hemolysis including a drop in Hb level to 3.5 g/dL, LDH of 12,460 U/L and bilirubin of 111 μmol/L. Renal failure ensued. Serological evaluation revealed RBC antibodies against all cells tested (including transfused units), a positive DAT with anti-C3d and anti-IgG, a positive eluate. No new allo-antibodies were detected, concluding to the presence of RBC auto-antibodies. Hb dropped to 2 g/dL and the patient's consciousness was mildly impaired. Then, he received 6 more units compatible with the known allo-antibodies, and was given steroids (iv methylprednisolone), a pulse of 1000 mg of iv cyclophosphamide and erythropoietin. Additional units were transfused. The patient clinical status gradually improved, Hb level rose up to 6 g/dL and he was discharged from the intensive care unit on day 30. Lymphocyte subsets increased on day 14 as well as natural killer (NK) cells which increased ten times (Figure 2A). IL-10 transcripts obtained from unstimulated peripheral blood mononuclear cells were quantified as already described.[13] They were over 900 copies at day 15 as compared to the level around 50 at day 32. The auto-antibodies finally disappeared. After this last DHTR episode, the avoidance of transfusion was strongly recommended.

Unfortunately, a new hip fracture occurred in 2007, responsible of a large hematoma with blood loss. A new hip replacement was required and transfusion absolutely necessary. To prevent a new DHTR, rituximab (1000 mg) was administered, 3 days before surgery and transfusion, and 7 days after the procedure (Figure 2A). Before surgery, serum contained anti-C, anti-Fya, and anti-S and the DAT was negative. 1,5 L of blood was lost during surgery, then 7 units similar to the units used before the 2005 DHTR were transfused. On days 5, 8, 10 and 20, sera remained compatible with samples of RBCs units received at day 0, the DAT and the eluate remained negative. Hb A rose up to 40% after transfusion and decreased slowly to reach 17% on day 21.

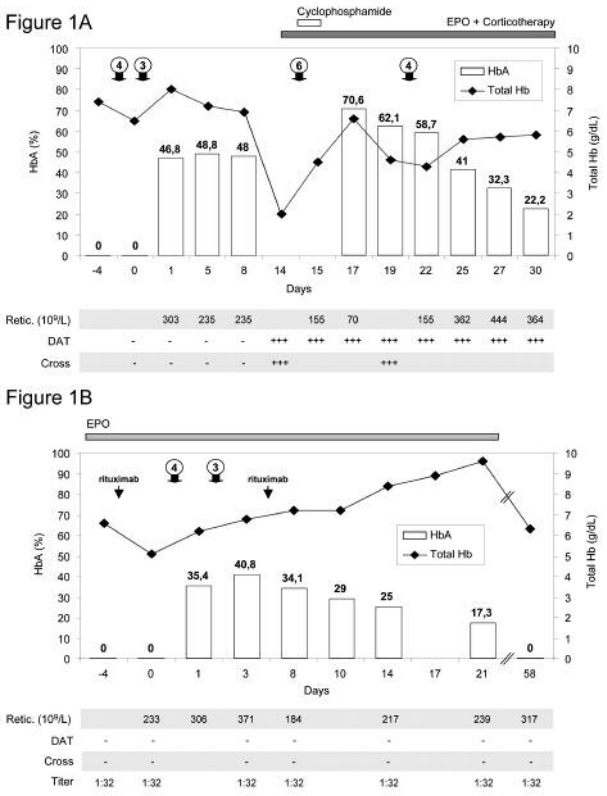

Figure 1. Clinical course of the episode of DHTR (A) and the episode of transfusion with rituximab prophylaxis (B). Numbers within circles indicate number of units transfused. Hb A is indicated in percentage and total Hb in g/dL. Absolute reticulocyte counts (10^9/L), intensity of DAT and results of crossmatches between serum and samples of the 7 first units transfused are indicated below the figure. Units transfused at day 0 and 1, compatible with all known allo-antibodies (cross negative) were well tolerated, Hb was stabilised and HbA raised up to 40% until day 8 whereas DAT and Cross remained negative. At day 14, Hb was below 2 g/dL reaching values lower than pre-transfusion level, % of HbA was not available, DAT was positive for IgG and complement, cross became strongly positive, suggesting auto-antibody production as no new allo-antibodies could be demonstrated. The 10 next units transfused were not compatible with the auto-antibodies as shown by positive crossmatch reactions. Administration of cyclophosphamide, corticosteroids and EPO was finally associated with an increase in total Hb and % of HbA. Decreased in reticulocyte count after day 14 probably accounts for the destruction of all RBCs by auto-antibodies, slowly corrected by treatment. In figure 1B, the first infusion of rituximab occurred 3 days before surgery, the second infusion 7 days after transfusion and surgery. Decreased in Hb level before transfusion was explained by the hematoma, then transfusion occurred, the Hb level increased up to 10 g/dL. No suppression of erythropoiesis was observed, as reticulocyte count remained stable. Titer of existing antibodies (anti-C, anti-Fya, anti-S) remained stable (1/32).

Treatment with rituximab resulted in a marked depletion of B cells. NK cells remained stable (Figure 2B). Titers of the existing allo-antibodies toward RBCs remained at 1/32. Total IgG, IgM and IgA determined by immunonephelometry stayed identical. After rituximab infusions, IL6 and TNFα sera levels were measured by a two-site sandwich immuno-assay (Immunlite; DPC) and an ELISA using a commercial kit Eli-pair (Diaclone, Besançon, France) respectively. They not reveal any cytokine-release syndrome, attesting the immediate safety of the procedure.[14] Three months after treatment, the patient was in good condition. The study protocol was approved by the Institutional Review Board of The Henri Mondor Hospital (N° 05-013). Written informed consent was obtained from the patient.

Discussion

Our data indicate that rituximab may prevent DHTR in SCD without causing significant side-effects. DHTR represents the main risk of RBC transfusion in these patients. Inflammatory factors produced during acute hemolysis can promote organ failure, and steroid therapy which can be helpful in controlling hemolysis and auto-antibody production may also promote a rebound of vaso-occlusive manifestations and lead to severe infections.[15] Then, after severe DHTR, clinicians became very precarious for the disease management which can however be far more damaging if RBC transfusion was not available. Prevention of allo-immunization and transfusion of matched RBCs compatible with all the known allo-antibodies are necessary but not sufficient to avoid DHTR, as shown by our case. In this setting, auto-immunization that appeared after transfusion played the main role as shown by the immuno-hematologic data and the peak of IL10 transcripts observed during the 2005 DHTR episode. IL10 acts as a critical mediator for auto-immunity and for RBC auto-antibody production.[16] It has also been shown that IL10 could activate NK cell cytotoxicity.[17] Furthermore, NK cells which were dramatically increased, acted probably as strong effectors cells through their FcγRIIIa receptors to destroy sensitised RBCs through ADCC. Then, we thought that rituximab could prevent DHTR for this patient, mainly by inhibiting development of auto-antibodies but also by attracting and binding Fcγ receptor-expressing effector cells,[18] such as monocytes/macrophages or NK cells, which in turn would diminish their antibody-dependent cytotoxicity effect towards sensitised RBCs. The choice of a *prophylactic* treatment rather than a curative treatment was based on the recurrence of the reaction in our patient (3 prior DHTR), the expected delay of rituximab action on B cells, and the absence of correlation in several auto-immune diseases between decline of auto-antibody levels and clinical benefit.[19] Furthermore, we thought that the potential action of rituximab to prevent antigen presentation and T help could also be beneficial in preventing auto-immune hemolysis as auto-antibody production is mainly elicited by prior allo-immunization. Even when transfused RBCs are perfectly compatible taking into account all the known developed allo-antibodies but also the main immunogenic antigens (which was the case in the 2005 episode), patient exposure to a rare antigen such as a low frequency antigen, cannot be eliminated, raising the possibility of a new allo-immunization able to elicit auto-immunization. On the other hand, rituximab has no effect on plasmocytes and was not used in this case to eliminate existing allo-

Figure 2A

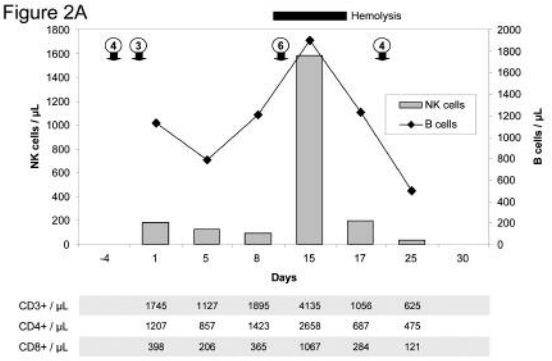

Figure 2B

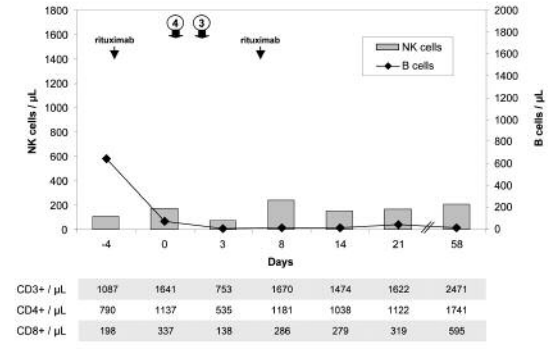

Figure 2. Lymphocytes subsets during the episode of DHTR (A) and the episode of transfusion with rituximab prophylaxis (B). Absolute B cells counts (CD19⁺) are indicated on the right y-axe and represented as curves, absolute NK cells (CD16⁺, CD56⁺) are indicated on the left y-axe and represented as histograms. Absolute count for other lymphocytes subsets (CD3⁺, CD4⁺, CD8+) are indicated below figure A and B for each episode. Prior the DHTR episode (Figure 2A), all populations were stable until day 8 and increased dramatically during the DHTR episode. In proportion, NK cells were the most elevated as they reached ten times their basal level at day 15. In figure 2B, the first infusion of rituximab occurred 3 days before surgery, the second infusion 7 days after transfusion and surgery. B cells (2B) dropped from the pre treatment values of 12% to values less than 0.5% 5 days after the first infusion. The other populations remained stable, except at day 3, because of blood loss and transfusion.

antibodies towards RBCs produced from previous allo-immunizations. Titers of pre-existing allo-antibodies towards RBCs (Figure 1B) as well as levels of IgG, IgA and IgM remained stable.

This observation shows for the first time that DHTR can be prevented. We believe that the use of rituximab should be considered when a new transfusion seems inevitable in patients with SCD and a prior history of life-threatening DHTR with production of auto-antibodies. It remains that since hyper haemolysis syndrome can also responds to therapy with short-term intravenous immunoglobulin and methylprednisolone which do not cause long term B-lymphocyte depletion, caution has to be advice in deciding this prevention, which could be considered as a second line approach when the other treatment failed. As for other autoimmune diseases, the best regimen (schedule and dosing) for the use of rituximab remains to be established. The next goal would be to bring evidence that rituximab can also prevent allo-immunization in order to provide a new transfusion strategy for SCD patients when compatible blood units are not available.

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References

1. Talano JA, Hillery CA, Gottschall JL, Baylerian DM, Scott JP. Delayed hemolytic transfusion reaction/hyperhemolysis syndrome in children with sickle cell disease. Pediatrics 2003; 111:e661-5.
2. McGlennan AP, Grundy EM. Delayed haemolytic transfusion reaction and hyperhaemolysis complicating peri-operative blood transfusion in sickle cell disease. Anaesthesia 2005; 60:609-12.
3. Syed SK, Sears DA, Werch JB, Udden MM, Milam JD. Case reports: delayed hemolytic transfusion reaction in sickle cell disease. Am J Med Sci 1996; 312:175-81.
4. Noizat-Pirenne F, Lee K, Pennec PY, Simon P, Kazup P, Bachir D, et al. Rare RHCE phenotypes in black individuals of Afro-Caribbean origin: identification and transfusion safety. Blood 2002; 100:4223-31.
5. King KE, Shirey RS, Lankiewicz MW, Young-Ramsaran J, Ness PM. Delayed hemolytic transfusion reactions in sickle cell disease: simultaneous destruction of recipients' red cells. Transfusion 1997; 37:376-81.
6. Petz LD. Bystander immune cytolysis. Transfus Med Rev 2006; 20:110-40.
7. Liu C, Marshall P, Schreibman I, Vu A, Gai W, Whitlow M. Interaction between terminal complement proteins C5b-7 and anionic phospholipids. Blood 1999; 93:2297-301.
8. Wang RH, Phillips G, Jr., Medof ME, Mold C. Activation of the alternative complement pathway by exposure of phosphatidylethanolamine and phosphatidylserine on erythrocytes from sickle cell disease patients. J Clin Invest 1993; 92:1326-35.
9. Aygun B, Padmanabhan S, Paley C, Chandrasekaran V. Clinical significance of RBC alloantibodies and autoantibodies in sickle cell patients who received transfusions. Transfusion 2002; 42:37-43.
10. Gonzalez CE, Pengetze YM. Post-transfusion purpura. Curr Hematol Rep 2005; 4:154-9.
11. Zecca M, Nobili B, Ramenghi U, Perrotta S, Amendola G, Rosito P, et al. Rituximab for the treatment of refractory autoimmune hemolytic anemia in children. Blood 2003 May 15;101(10):3857-61.
12. Pescovitz MD. Rituximab, an anti-cd20 monoclonal antibody: history and mechanism of action. Am J Transplant. 2006; 6:859-66.
13. Ansart-Pirenne H, Zeliszewski D, Lee K, Martin-Blanc S, Rouger P, Noizat-Pirenne F. Identification of immunodominant alloreactive T-cell epitopes on the Jka red blood cell protein inducing either Th1 or Th2 cytokine expression. Blood 2004; 104:3409-10.
14. Winkler U, Jensen M, Manzke O, Schulz H, Diehl V, Engert A. Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8). Blood 1999; 94:2217-24.
15. Bernini JC, Rogers ZR, Sandler ES, Reisch JS, Quinn CT,

Buchanan GR. Beneficial effect of intravenous dexamethasone in children with mild to moderately severe acute chest syndrome complicating sickle cell disease. Blood 1998; 92:3082-9.
16. Fagiolo E. Immunological tolerance loss vs. erythrocyte self antigens and cytokine network disregulation in autoimmune hemolytic anaemia. Autoimmun Rev 2004; 3:53-9.
17. Mocellin S, Panelli M, Wang E, Rossi CR, Pilati P, Nitti D, et al. IL-10 stimulatory effects on human NK cells explored by gene profile analysis. Genes Immun 2004; 5:621-30.
18. Taylor RP, Lindorfer MA. Drug insight: the mechanism of action of rituximab in autoimmune disease--the immune complex decoy hypothesis. Nat Clin Pract Rheumatol 2007; 3:86-95.
19. Sabahi R, Anolik JH. B-cell-targeted therapy for systemic lupus erythematosus. Drugs 2006; 66:1933-48.