Septic Episodes in a Premature Infant After In Utero Exposure to Rituximab

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Rituximab is an increasingly used immunotherapeutic agent for women of reproductive age for treatment of autoimmune diseases, leukemias, and lymphomas. Rituximab is a chimeric monoclonal antibody that targets B-cell surface antigen CD20 and can cross the placenta. Current evidence of the impact of this medication on the developing fetus is limited, but there is little to suggest that fetal exposure to this medication places an infant at increased risk of immunosuppression and subsequent infection. Here we report a case of in utero rituximab exposure that was associated with 2 severe septic episodes with *Enterococcus faecalis*, in a premature infant of 29 weeks' gestational age with a birth weight of 820 g. The patient had a critically depressed B-lymphocyte subset of 10% and undetectable immunoglobulin (Ig)G, IgM, and IgA levels at 37 weeks' postmenstrual age. Interestingly, both episodes of sepsis coincided with transition from donor human milk to formula feeds. She was treated with intravenous immunoglobulin, antibiotics, and donor human milk. We postulate that placental transfer of rituximab, prematurity, and the low levels of protective maternal antibodies increased the susceptibility of this patient to sepsis by *E faecalis*, a resident of the normal gut flora, whereas the secretory IgA in donor human milk may have played a protective role.

Rituximab is a chimeric monoclonal antibody directed against B-cell surface antigen CD20. Rituximab is approved for treatment of certain types of non-Hodgkin lymphoma (NHL) and rheumatoid arthritis. Rituximab administration results in depletion of peripherally circulating CD20-expressing B cells. As a monoclonal antibody, rituximab contains an immunoglobulin (Ig) G1κ construct and can cross the placenta.[1] Current evidence of the impact of this medication on the developing fetus is limited, but there is little to suggest that fetal exposure to this medication places an infant at increased risk of immunosuppression and subsequent infection.[2–7] Here we report a case of in utero rituximab exposure in a premature infant whose mother was undergoing treatment of NHL with chemotherapy and immunotherapy. The infant's hospital course was notable for 2 separate septic episodes with *Enterococcus faecalis* in association with critically low numbers of circulating B cells and low levels of serum immunoglobulins. Interestingly, both episodes of sepsis coincided with transition from donor human milk to formula feeds. She was treated with intravenous immunoglobulin (IVIg), antibiotics, and donor human milk. We present this case to highlight an important adverse effect of in utero rituximab exposure on the immune system of the fetus, especially in the setting of premature birth. Our case also underscores the protective effect of donor human milk, presumably due to secretory IgA (sIgA), in this immunocompromised host.

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CASE REPORT

Our patient, a female infant, was born by cesarean delivery at 29 weeks and 2 days' gestation for worsening gestational hypertension and maternal renal function in the setting of NHL. The pregnancy was complicated by maternal diagnosis of NHL, lymphoplasmacytic type, during the first trimester, with chemotherapy and immunotherapy administered between weeks 14 and 27 of gestation. The infant emerged limp with poor respiratory effort. She demonstrated good response to positive pressure ventilation and was admitted to the NICU on continuous positive airway pressure. Admission examination was unremarkable aside from the presence of symmetric intrauterine growth restriction (birth weight 820 g, 3rd percentile; length 36 cm, 17th percentile; head circumference 24.5 cm, 6th percentile) and mild respiratory distress. Complete blood count at birth showed a white blood cell (WBC) count of 5.22 K/μL (59% neutrophils, 33% lymphocytes, 0% bands), a hematocrit of 44.3%, and a platelet count of 234 K/μL. The mother's antenatal laboratory screens ruled out risk for syphilis, HIV, and rubella infections, and the infant's urine was negative for cytomegalovirus. The infant was transitioned from continuous positive airway pressure to room air over the course of 8 days. She was fed with donor human milk and then transitioned to a formula by the 23rd day of life.

On day of life 24 (postmenstrual age [PMA] 33 weeks and 0 days), the patient experienced clinical decompensation, with significant bradycardic events and hypoxia, prompting intubation and initiation of high-frequency ventilation. Laboratory studies demonstrated a WBC count of 9.14 K/μL (66% neutrophils, 36% lymphocytes, 10% bands). Blood and urine cultures were sent and empirical antibiotic coverage with vancomycin and zosyn was started due to concerns for an intra-abdominal source of infection. Enteral feeds were replaced with total parenteral nutrition. Lumbar puncture for cerebrospinal fluid (CSF) studies was deferred because of the patient's tenuous clinical status. Bacteremia persisted on repeat blood cultures through day 2 of illness despite therapeutic vancomycin levels. Both blood and urine cultures grew *E faecalis*, and antibiotic coverage was transitioned to ampicillin with gentamicin. Susceptibilities of the isolates included susceptibility to ampicillin, high-level gentamicin (no interpretation for standard gentamicin), and vancomycin; the only demonstrated resistances were to rifampin and oxacillin. A workup for a potential septic nidus, including echocardiogram, abdominal ultrasound, and brain ultrasound, was unremarkable. Repeat blood cultures 48 hours after commencement of antibiotics were negative. The patient demonstrated rapid clinical improvement on treatment, with ability to restart feeds with donor human milk on day of life 29. She was again transitioned from donor human milk to formula on day of life 47. As it was impossible to prove the absence of meningitis given the lack of CSF studies, the decision was made to treat her for presumed meningitis with a 3-week course of antibiotics, which was completed with ampicillin and gentamicin on day of life 48.

However, on day of life 50 (PMA 36 weeks and 5 days), the patient had another episode of clinical decompensation, with recurrent apnea, bradycardia, hypoxia, lethargy, and temperature instability. She again required intubation and support with synchronized intermittent mandatory ventilation. Complete blood count demonstrated a WBC count of 8.98 K/μL (49% neutrophils, 49% lymphocytes, 0% bands). Blood and CSF cultures were sent and broad-spectrum antibiotic coverage with nafcillin and gentamicin was started. Lumbar puncture revealed cloudy CSF with a WBC count of 7425 cells/μL (81% neutrophils), red blood cell count of 756 cells/μL, total protein of 1046 mg/dL, and glucose of 28 mg/dL with simultaneous serum glucose of 111 mg/dL. Given concern for antibiotic resistance in the context of the unusual clinical course, antibiotic coverage was empirically changed to vancomycin and ceftazidime (while cefotaxime was unavailable), pending culture results. Both blood and CSF cultures grew *E faecalis*. Once the organism was identified, antibiotic coverage was changed to ampicillin and cefotaxime (which had become available), and these antibiotics were continued for a total of 3 weeks for treatment of sepsis and meningitis. Once again, the patient's clinical status improved and blood cultures became sterile within 24 hours of antibiotic treatment. Susceptibilities of the isolates were the same as before, including susceptibility to ampicillin, high-level gentamicin (no interpretation for standard gentamicin), and vancomycin; the only demonstrated resistance was to rifampin, with oxacillin not assessed. No further testing was performed to confirm that this was the same organism as before. Further workup was pursued in search for a persistent nidus of infection. However, the results were unremarkable, including head ultrasound, echocardiogram, abdominal ultrasound, Doppler ultrasound of previous peripherally inserted central catheter site, spinal ultrasound, and brain MRI.

On further review of maternal history, it was noted that the patient's mother had an unusual form of NHL: IgG λ lymphoplasmacytic lymphoma. Her laboratory studies showed low serum IgG and IgM levels in the setting of this form of NHL (Table 1), and she had been treated with a chemotherapeutic and immunotherapeutic regimen

TABLE 1 Patient and Maternal Immunoglobulin Levels

	Patient	Mother
IgG	<40[a] mg/dL (64–237 mg/dL)	279[a] mg/dL (700–1600 mg/dL)
IgM	<25 mg/dL (4.9–46.7 mg/dL)	38[a] mg/dL (70–400 mg/dL)
IgA	<5 mg/dL (1.1–8.3 mg/dL)	70 mg/dL (40–230 mg/dL)

Patient values at 37 weeks and 0 days PMA. Normal limits in parentheses provided for gestational and chronological age.[8] Maternal studies from 2 days before patient's birth.
[a] Value outside of normal range.

that consisted of rituximab, cyclophosphamide, and prednisone, which was started in the second trimester and given as 5 courses spaced in 3-week intervals. For concern that rituximab, a monoclonal antibody to CD20, might have resulted in fetal B-cell ablation, further immunologic evaluation was performed on our patient. The immunologic workup, performed at 37 weeks and 0 days PMA, revealed a depressed B-cell subset of 10% (normal 20%–30%). IgG (<40), IgA (<5), and IgM (<25) levels were all below the detection limit of the assay (Table 1). Based on these results, IVIg treatment (1 g/kg/d in 2 aliquots) was provided, and feeds were resumed with donor human milk. Repeat immunoglobulin levels were performed 3 weeks later (PMA 39 weeks and 3 days), demonstrating a low but improved IgG level of 444. A second course of IVIg was given with a plan to continue monthly IVIg infusions and donor human milk up to 6 months of life.

The patient's subsequent NICU course was uneventful. She was discharged from the hospital after a 2.5-month admission. Her neurologic studies, including brain MRI and neurologic examination at the time of discharge from the hospital, were normal, and she was scheduled to follow-up with subspecialties that included Allergy/Immunology and Neurology.

DISCUSSION

To the best of our knowledge, this is the first case report that suggests a clinically significant immunocompromised state with 2 episodes of sepsis in a preterm infant subsequent to in utero exposure to rituximab with B-cell and immunoglobulin depletion. In a previous case report, Klink et al[2] demonstrated complete depletion of neonatal B lymphocytes in a term infant after in utero exposure to rituximab without any adverse clinical consequences. The case we describe here highlights the role of prematurity in potentiating the adverse effects of in utero rituximab exposure. It is well known that the fetus obtains nearly all of its immunoglobulins via transplacental transfer of maternal IgG.[1] This transfer is mediated by a specific receptor, and active transport starts early in fetal development, increasing progressively until complete gestation. Normative data on IgG levels in preterm newborns have shown that this population has not yet received sufficient titers of protective antibodies, with ∼400 mg/dL IgG detected at 32 weeks' gestation and most antibodies transferred after the 34th week of gestation.[9] Preterm newborns are subsequently at increased risk of infection at baseline, with the reported rates of late-onset sepsis in infants born at 29 to 32 weeks of gestation being ∼10%.[10] It also should be noted that our patient's mother had an abnormal serum immunoglobulin profile, with low serum IgG and IgM levels secondary to IgG-restricted lymphoplasmacytic lymphoma (Table 1). Thus, in addition to placental transfer of rituximab with direct effects on fetal B cells, low maternal immunoglobulin levels before and exacerbated by rituximab treatment also could have negatively affected the immunoglobulin transfer across the placenta in our patient. The contribution of the underlying biology of maternal disease is also a possibility.

Rituximab is currently a Food and Drug Administration Pregnancy Category C medication, with adverse effects demonstrated in animal reproduction studies, but inadequate well-controlled human studies.[11] Although the potential benefits of rituximab may warrant its use despite potential risks, it is recommended by the Food and Drug Administration to avoid pregnancy for a minimum of 12 months after therapy. As a monoclonal antibody with a G1κ construct, it has the ability to cross the placenta.[1] Few reports are available with regard to the outcomes of infants with in utero exposure to rituximab. Most of these describe reassuring outcomes, with rare cases of transient and inconsequential B-cell suppression.[3–6] The largest series was recently reported by Chakravarty et al,[7] who described the outcomes of 153 cases of fetal rituximab exposure. In this series, of 90 live births, 22 were preterm, 1 died in the neonatal period, and 2 demonstrated congenital malformations.

Although most of these cases were confounded by concomitant use of teratogenic medications, this study reported that 11 of 90 live births developed hematologic abnormalities, including B-cell

suppression. However, none of these cases of hematologic abnormalities were associated with infections. The 3 presumed cases of infections were associated with fever, bronchiolitis, and chorioamnionitis, and there was 1 case of cytomegalovirus hepatitis. Thus, there were no documented cases of bacterial sepsis, even in preterm infants. However, none of these infants were born at <30 weeks' gestation.

It additionally should be noted that the contribution of maternal rituximab to our patient's symmetric growth restriction is unclear. No such association with rituximab exposure has been described in the literature, and the etiology of growth restriction secondary to the steroids or cyclophosphamide of this mother's oncologic regimen or her hypertension is possible.[12,13] The contribution of these additional maternal medications to our patient's immunosuppression, however, seems less likely. Immunosuppression is not a recognized complication of maternal prednisone use, and normal neonatal immunoglobulin levels have been previously described in the context of maternal prednisone use.[14] Cyclophosphamide can cause fetal malformations and miscarriages[15] and has been linked to the development of cancer in mice[16]; however, with regard to immune function, only mild asymptomatic neutropenia has been described.[17]

The etiology of most enterococcal infections is thought to be via translocation of endogenous bacteria through the epithelial cells of the intestine.[18] The virulence of *Enterococcus* species is influenced by its ability to colonize the gastrointestinal tract, as well as its ability to adhere to a range of extracellular matrix proteins and epithelia.[19] Hendrickx et al[20] described the interaction of *Enterococcus faecium* with sIgA, agglutinating with this immunoglobulin and other proteins to maintain spatial segregation from the intestinal wall. The preservation of sIgA in pasteurized donor milk has been described, with protein retention ranging from 70% to 80%.[21,22] This protective agglutination may be the etiology of our patient's apparent dependence on the sIgA from donor human milk to prevent transmural migration of *Enterococcus*. Additionally, the role of IgG depletion in our patient's enterococcal sepsis may be secondary to dependence on opsonization for clearance of this bacteria. Such enterococcal isolates resistant to neutrophil and macrophage phagocytosis have been previously described.[23]

CONCLUSIONS

We describe a case of 2 episodes of sepsis in a premature infant potentially due to compromised humoral immunity after in utero exposure to rituximab. Based on this case, we suggest that infants of mothers treated with rituximab, especially those born prematurely, should be monitored closely for clinical signs of infection with a lower threshold for evaluation, including B-cell numbers and serum immunoglobulin levels, if there is clinical concern. Human milk, even after pasteurization, should be the preferred feeds in these infants, as it appears to contain adequate levels of sIgA for protection against infections with the gut flora.

ABBREVIATIONS

CSF: cerebrospinal fluid
Ig: immunoglobulin
IVIg: intravenous immunoglobulin
NHL: non-Hodgkin lymphoma
PMA: postmenstrual age
sIg: secretory immunoglobulin
WBC: white blood cell

REFERENCES

1. Saji F, Samejima Y, Kamiura S, Koyama M. Dynamics of immunoglobulins at the feto-maternal interface. *Rev Reprod*. 1999;4(2):81–89
2. Klink DT, van Elburg RM, Schreurs MW, van Well GT. Rituximab administration in third trimester of pregnancy suppresses neonatal B-cell development. *Clin Dev Immunol*. 2008;2008:271363
3. Martínez-Martínez MU, Baranda-Cándido L, González-Amaro R, Pérez-Ramírez O, Abud-Mendoza C. Modified neonatal B-cell repertoire as a consequence of rituximab administration to a pregnant woman. *Rheumatology (Oxford)*. 2013;52(2):405–406
4. Ojeda-Uribe M, Gilliot C, Jung G, Drenou B, Brunot A. Administration of rituximab during the first trimester of pregnancy without consequences for the newborn. *J Perinatol*. 2006;26(4):252–255
5. Pendergraft WF III, McGrath MM, Murphy AP, et al. Fetal outcomes after rituximab exposure in women with autoimmune vasculitis. *Ann Rheum Dis*. 2013;72(12):2051–2053
6. Friedrichs B, Tiemann M, Salwender H, Verpoort K, Wenger MK, Schmitz N. The effects of rituximab treatment during pregnancy on a neonate. *Haematologica*. 2006;91(10):1426–1427
7. Chakravarty EF, Murray ER, Kelman A, Farmer P. Pregnancy outcomes after maternal exposure to rituximab. *Blood*. 2011;117(5):1499–1506
8. Ballow M, Cates KL, Rowe JC, Goetz C, Desbonnet C. Development of the immune system in very low birth weight (less than 1500 g) premature infants: concentrations of plasma immunoglobulins and patterns of infections. *Pediatr Res*. 1986;20(9):899–904
9. Landor M. Maternal-fetal transfer of immunoglobulins. *Ann Allergy Asthma Immunol*. 1995;74(4):279–283, quiz 284
10. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics*. 2002;110(2 pt 1):285–291

11. Boothby LA, Doering PL. FDA labeling system for drugs in pregnancy. *Ann Pharmacother*. 2001;35(11):1485–1489

12. Lunghi L, Pavan B, Biondi C, et al. Use of glucocorticoids in pregnancy. *Curr Pharm Des*. 2010;16(32):3616–3637

13. Østensen M, Khamashta M, Lockshin M, et al. Anti-inflammatory and immunosuppressive drugs and reproduction. *Arthritis Res Ther*. 2006;8(3):209

14. Cederqvist LL, Merkatz IR, Litwin SD. Fetal immunoglobulin synthesis following maternal immunosuppression. *Am J Obstet Gynecol*. 1977;129(6):687–690

15. Lannes G, Elias FR, Cunha B, et al. Successful pregnancy after cyclophosphamide therapy for lupus nephritis. *Arch Gynecol Obstet*. 2011;283(suppl 1):61–65

16. Comish PB, Drumond AL, Kinnell HL, et al. Fetal cyclophosphamide exposure induces testicular cancer and reduced spermatogenesis and ovarian follicle numbers in mice. *PLoS One*. 2014;9(4):e93311

17. Ponticelli C, Moroni G. Immunosuppression in pregnant women with systemic lupus erythematosus. *Expert Rev Clin Immunol*. 2015;11(5):549–552

18. Franz CM, Holzapfel WH, Stiles ME. Enterococci at the crossroads of food safety? *Int J Food Microbiol*. 1999;47(1–2):1–24

19. Fisher K, Phillips C. The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology*. 2009;155(pt 6):1749–1757

20. Hendrickx AP, Top J, Bayjanov JR, et al. Antibiotic-driven dysbiosis mediates intraluminal agglutination and alternative segregation of *Enterococcus faecium* from the intestinal epithelium. *MBio*. 2015;6(6):e01346-15

21. Czank C, Prime DK, Hartmann B, Simmer K, Hartmann PE. Retention of the immunological proteins of pasteurized human milk in relation to pasteurizer design and practice. *Pediatr Res*. 2009;66(4):374–379

22. Ford JE, Law BA, Marshall VM, Reiter B. Influence of the heat treatment of human milk on some of its protective constituents. *J Pediatr*. 1977;90(1):29–35

23. Rakita RM, Quan VC, Jacques-Palaz K, et al. Specific antibody promotes opsonization and PMN-mediated killing of phagocytosis-resistant *Enterococcus faecium*. *FEMS Immunol Med Microbiol*. 2000;28(4):291–299

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